

**STUDIES ON MORPHO-PHYSIOLOGICAL,
BIOCHEMICAL AND MOLECULAR MECHANISM OF
FLOODING TOLERANCE IN PULSES**

*Thesis submitted in part fulfilment of the requirements for the
degree of **Doctor of Philosophy (Agriculture) in Crop Physiology**
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By

V. RAVICHANDRAN

I.D.No. 99-808-002

**DEPARTMENT OF CROP PHYSIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE - 641 003**

2002

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR MECHANISM OF FLOODING TOLERANCE IN PULSES" submitted in part fulfilment of the requirements for the degree of **Doctor of Philosophy (Agriculture) in Crop Physiology** to the Tamil Nadu Agricultural University, Coimbatore, is a bonafide record of research work carried out by **Mr. V. RAVICHANDRAN** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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(Dr.G. PATHMANABHAN)
CHAIRMAN

APPROVED BY

Chairman:


(Dr.G. PATHMANABHAN)


Members:


(Dr.M. THANGARAJ)


(Dr.T.N. BALASUBRAMANIAN)

(Dr.A.R. MUTHIAH)

Date : 6.9.2002 EXTERNAL EXAMINER:


(G.V. HANUMANTHA RAO)

Abstract

ABSTRACT

STUDIES ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR MECHANISMS OF FLOODING TOLERANCE IN PULSES

By

V. RAVICHANDRAN

Degree : Doctor of Philosophy (Agriculture) in Crop Physiology

Chairman : **Dr.G. PATHMANABHAN, Ph.D.,**

Professor

Department of Crop Physiology

Tamil Nadu Agricultural University

Coimbatore-641 003.

Year : **2002**

Studies were conducted at Tamil Nadu Agriculture University, Coimbatore during the year 1999 - 2002 to screen different pulse varieties for flooding tolerance through Triphenyl Tetrazolium Chloride (TTC) reduction method, Electrical Conductivity (EC) test, Pressure Chamber Technique and root Alcohol Dehydrogenase (ADH) activity tests and also to investigate the morpho-physiological, biochemical and molecular mechanisms of flooding tolerance. The possibility of chemical manipulation for induction of flooding tolerance through different chemicals and plant growth regulators was also investigated.

Lab screening studies revealed that CO 4 cowpea, CO 4 green gram and CO 1 soybean showed tolerance behaviour to flooding due to higher TTC reducing ability, less ions efflux, high leaf water potential and root ADH activity among the pulse cultivars tested.

In pot culture study, among the three pulses, CO 4 cowpea, CO 4 green gram and CO 1 soybean had altered their morphological features and reduced their growth attributes like plant height, leaf area, specific Leaf Weight (SLW) and Specific Leaf

Area (SLA) under flooded condition. Root parameters like root numbers and root length had increased for tolerant varieties.

Leaf gas exchange parameters like photosynthesis, transpiration rates and leaf water potential decreased with the flooding but the stomatal resistance was increased. The leaf chlorophyll content and chlorophyll fluorescence ratio were most affected by longer duration of flooding.

The biochemical constituents such as soluble protein and total carbohydrate content were reduced under flooding but accumulation of root anaerobic polypeptides was more in flooded roots. The root nodulation, nitrogenase activity and leghaemoglobin contents were affected mostly during flooding at flowering stage, which further inhibits nitrogen fixation in pulses.

The activity of free radical scavenging enzymes *viz.*, catalase, peroxidase and superoxide dismutase increased in tolerant varieties under flooding which revealed, their strong association with decreasing lipid peroxidation and membrane damage.

Flooding had increased the activity of Alcohol Dehydrogenase (ADH) and Pyruvate Decarboxylase (PDC) enzymes for anoxia survival in pulses. Maximum level of activity was achieved under shorter duration of flooding (5 days) in tolerant pulse varieties. Ultimately Malate Dehydrogenase (MDH) also increased in tolerant varieties for re-routing the ethanol pathway.

The protein profiles of leaf and roots, isozymes banding pattern of peroxidase showed distinct differences between control and flooded pulse varieties. Morphological and anatomical studies revealed that increased ethylene production content induced the adventitious root formation, stem hypertrophy and lysigenous aerenchyma development under flooded condition. These changes had strengthened adaptive significance for pulses under flooded condition.

Flooding at vegetative, flowering and pod filling stages decreased total dry matter accumulation in all the pulse varieties studied, with significant decrease in yield components *viz.*, number of flowers and pods, fertility coefficient, seeds weight, seed number, seed yield per plant. The effect of flooding at flowering and pod filling was more detrimental than at vegetative stage.

The results on the induction of flooding tolerance by exogenous application of different growth regulators and chemicals revealed that deleterious effect of flooding on various yields and yield components could partially be alleviated. Best among the ameliorants were brassino steroids (0.1 ppm) and salicylic acid (100 ppm) as foliar sprays at flowering and pod filling stages. In green gram, the varieties CO 4 and VBN 1 differed significantly at flood condition for seed yield. Thus, CO 4 gave highest seed yield by the application of ameliorants at flowering and pod filling stages during flood condition.

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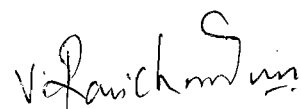
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CONTENTS

Chapter No.	Title	Page No.
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	5
III.	MATERIALS AND METHODS	43
IV.	EXPERIMENTAL RESULTS	68
V.	DISCUSSION	194
VI.	SUMMARY	222
	REFERENCES	258

LIST OF TABLES

Table No.	Title	Page No.
1	Details of pulse crops selected for study	44
2	Soil properties of the experimental field	48
3	Weather data for the cropping period	65
4	Screening of pulses for flooding tolerance by Triphenyl Tetrazolium Chloride reduction method, Electrical Conductivity (EC) Test, Pressure Chamber method (Water potential) and root Alcohol Dehydrogenase (ADH) activity	69
5	Effect of flood stress on plant height (cm) in pulses at different growth stages	80
6	Effect of flood stress on root length (cm) in pulses at different growth stages	82
7	Effect of flood stress on root number in pulses at different growth stages	85
8	Effect of flood stress on nodule number in pulses at different growth stages	87
9	Effect of flood stress on nodule dry weight (mg plant ⁻¹) in pulses at different growth stages	90
11	Effect of flood stress on nitrogenase activity ($\mu\text{m C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$) in pulses at different growth stages	92
10	Effect of flood stress on leghaemoglobin content (mg g ⁻¹ of dry nodule) in pulses at different growth stages	95
12	Effect of flood stress on leaf area (cm ² plant ⁻¹) in pulses at different growth stages	99

13	Effect of flood stress on Specific leaf weight (SLW) (mg cm^{-2}) in pulses at different growth stages	102
14	Effect of flood stress on Specific leaf area (SLA) ($\text{cm}^2 \text{g}^{-1}$) in pulses at different growth stages	104
15	Effect of flood stress on total dry matter accumulation (g plant^{-1}) in pulses at different growth stages	106
16	Effect of flood stress on photosynthetic rate ($\mu \text{ moles CO}_2 \text{ m}^{-1} \text{ s}^{-1}$) in pulses at different growth stages	109
17 _x	Effect of flood stress on transpiration rate ($\mu \text{ mole H}_2\text{O m}^{-1} \text{ s}^{-1}$) in pulses at different growth stages	112
18 _x	Effect of flood stress on stomatal resistance (s cm^{-1}) in pulses at different growth stages	114
19	Effect of flood stress on leaf water potential ($-\text{MPa}$) in pulses at different growth stages	116
20 _x	Effect of flood stress on chlorophyll 'a' content (mg g^{-1}) in pulses at different growth stages	118
21 _x	Effect of flood stress on chlorophyll 'b' content (mg g^{-1}) in pulses at different growth stages	120
22 _x	Effect of flood stress on Total chlorophyll content (a/b ratio) (mg g^{-1}) in pulses at different growth stages	122
23	Effect of flood stress on chlorophyll fluorescence - variable fluorescence (fv) in pulses at different growth stages	124
24	Effect of flood stress on chlorophyll fluorescence - maximum fluorescence (fm) in pulses at different growth stages	126
25	Effect of flood stress on chlorophyll fluorescence ratio (fv/fm) in pulses at different growth stages	129
26 _x	Effect of flood stress on leaf soluble protein content (mg g^{-1}) in pulses at different growth stages	132

27	Effect of flood stress on root anaerobic protein content (mg g^{-1}) in pulses at different growth stages	134
28	Effect of flood stress on leaf starch (mg g^{-1}) in pulses at different growth stages	137
29	Effect of flood stress on total carbohydrates content (mg g^{-1}) in pulses at different growth stages	139
30	Effect of flood stress on leaf nitrate reductase activity (μ moles NO_2 produced $\text{g}^{-1} \text{h}^{-1}$) in pulses at different growth stages	142
31	Effect of flood stress on Alcohol Dehydrogenase (ADH) activity (μ moles NADH oxidised $\text{min}^{-1} \text{mg}^{-1}$ of sample) in pulses at different growth stages	144
32	Effect of flood stress on Pyruvate Decarboxylase (PDC) activity (enzyme units g^{-1} fresh weight) in pulses at different growth stages	146
33	Effect of flood stress on Malate Dehydrogenase (MDH) activity (μ moles NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ of extract) in pulses at different growth stages	148
34.	Effect of flood stress on root ethylene content (μ moles) in pulses at different growth stages	151
35	Effect of flood stress on peroxidase activity (enzyme units $\text{l}^{-1} \text{h}^{-1}$) in pulses at different growth stages	154
36	Effect of flood stress on catalase activity ($\times 10^4$ enzyme units $\text{g}^{-1} \text{min}^{-1}$) in pulses at different growth stages	157
37	Effect of flood stress on Superoxide Dismutase (SOD) activity (enzyme units $\text{mg protein}^{-1} \text{min}^{-1}$) in pulses at different growth stages	159
38	Effect of flood stress on number of flowers per plant in pulses at different growth stages	161

39	Effect of flood stress on number of pods per plant in pulses at different growth stages	164
40	Effect of flood stress on fertility co-efficient in pulses at different growth stages	161
41	Effect of flood stress on number of seeds per plant in pulses at different growth stages	170
42	Effect of flood stress on 100 seed weight (g) in pulses at different growth stages	172
43	Effect of flood stress on seed yield per plant (g) in pulses at different growth stages	174
44	Study of SDS-PAGE leaf and root protein profiles in pulses under flooded conditions	178
45	Effect of ameliorants on number of flowers per plant and number of pods per cluster in green gram varieties	183
46	Effect of ameliorants on number of pods per plant and fertility coefficient in green gram varieties	186
47	Effect of ameliorants on number of seeds per plant and hundred seed weight (g) in green gram varieties	189
48	Effect of ameliorants on seed yield (g plant ⁻¹) and biological yield (kg per hectare) in green gram varieties	190
49	Effect of ameliorants on seed yield (kg per hectare) and harvest index in green gram varieties	192

LIST OF FIGURES

Table No.	Title	Page No.
1.	Screening of pulses for flooding tolerance by Triphenyl Tetrazolium Chloride (TTC) reduction method	70
2.	Screening of pulses for flooding tolerance by Electrical Conductivity (EC) Test	73
3.	Screening of pulses for flooding tolerance by Leaf water potential (-MPa)	75
4.	Screening of pulses for flooding tolerance by Alcohol Dehydrogenase (ADH) activity	77
5.	Effect of flood stress on root length (cm)	83
6.	Effect of flood stress on nodule number	88
7.	Effect of flood leghaemoglobin content (mg g^{-1} of dry nodule)	93
8.	Effect of flood stress on nitrogenase activity ($\mu\text{m C}_2\text{H}_2 \text{ plant}^{-1} \text{ hr}^{-1}$)	96
9.	Effect of flood stress on leaf area ($\text{cm}^2 \text{ plant}^{-1}$)	100
10.	Effect of flood stress on total dry matter accumulation (g plant^{-1})	107
11.	Effect of flood stress on photosynthetic rate ($\mu \text{ moles CO}_2 \text{ m}^{-1} \text{ s}^{-1}$)	110
12.	Effect of flood stress on chlorophyll fluorescence ratio (fv/fm)	130
13.	Effect of flood stress on root protein (mg g^{-1})	135
14.	Effect of flood stress on Malate Dehydrogenase (MDH) activity ($\mu \text{ moles NADH oxidized min}^{-1} \text{ mg}^{-1}$ of extract)	149

15.	Effect of flood stress on root ethylene content (μ moles)	152
16.	Effect of flood stress on number of flowers per plant	162
17.	Effect of flood stress on fertility coefficient ratio	168
18.	Effect of flood stress on seed yield per plant (g)	175
19.	Effect of ameliorants on number of flowers per plant under 5 days flooding in greengram	184
20.	Effect of ameliorants on number of pods per plant under 5 days flooding in greengram	187
21.	Effect of ameliorants on seed yield (kg/ha) under 5 days flooding in greengram	193

LIST OF PLATES

Plate No.	Title	Page No.
1.	View of pot culture experiment	251
2.	Chemical manipulation for induction of flooding tolerance in green gram	252
3.	Formation of adventitious roots and stem cracking in pulses	253
4.	Formation of adventitious roots and floating roots in pulses under flooded conditions	256
5.	Microtome section of adventitious roots of pulses grown in control and flooded conditions	259
6.	Leaf and root peroxidase isozyme pattern of flood tolerant and susceptible pulse varieties	262
7.	Leaf and root protein profiles (SDS-PAGE) of pulses under control and flooded conditions	265

Abbreviations

LIST OF ABBREVIATIONS

ANPs	Anaerobic polypeptides
BR	Brassinosteroid
APS	Ammonium per sulphate
CAT	Catalase
E	Transpiration rate
FCRD	Factorial completely randomised design
FRBD	Factorial randomised block design
f.w.	Fresh weight
HI	Harvest Index
H ₂ O ₂	Hydrogen peroxide
KDa	Kilo dalton
MPa	Mega pascal
NRase	Nitrate reductase
NBT	Nitro blue tetrazolium
POX	Peroxidase
ppm	Parts per million
rs	Stomatal resistance
RM	Relative mobility
SA	Salicylic acid
SOD	Superoxide dismutase
SDS-PAGE	Sodium dedecyl sulphate - polyacrylamide gel electrophoresis
TEMED	N , N, N', N', - Tetramethyl ethylene diamine
SLW	Specific leaf weight
SLA	Specific leaf area
TDMA	Total Dry Matter Accumulation
g	gram

%	Per cent
ha	Hectare
BA	Benzyl adenine
GA	Gibberellic acid
mmhos	Millimhos
h	hour
Kg	Kilogram
mg	Milligram
TTC	Triphenyl Tetrazolium Chloride (TTC)
EC	Electrical conductivity
ADH	Alcohol Dehydrogenase
MDH	Malate Dehydrogenase
PDC	Pyruvate Decarboxylase
C ₂ H ₄	Ethylene
Pn	Photosynthetic rate
fr	variable fluorescence
fm	maximum fluorescence
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide reduced
EDTA	Ethylene Diamine Tetra Acetic acid

Introduction

CHAPTER I

INTRODUCTION

Pulses gained importance in the global agriculture for their high protein content and also for their inherent capability of fixing atmospheric nitrogen through symbiotic bacteria. It includes a variety of crop species and they belong to the sub family papilionaceae of the family leguminosae. Among them pigeonpea or redgram (*Cajanus cajan* L.), chickpea or bengal gram (*Cicer arietinum* L.) mungbean or greengram (*Vigna radiata* L.) (Wilczek), cowpea (*Vigna unguiculata* (L) Walp. soybean (*Glycine max* (L.) Merr.) and black gram (*Vigna mungo* (L.) Hepper) are the major pulses in India grown either as pure crop or as intercrop in an area of about 20 million ha. with a total production of about 11.06 million tonnes (2001). The productivity of pulses has increased from 476 kg per ha. during 1970-75 to 553 kg per ha. during 2000-2001.

Pulses account for roughly one fifth of total area under food grain crops and contribute about one twelfth of total food production of the country. It is estimated that the country's demand of total pulses would be around 30 million tones by 2020 AD when the population of the country will be nearly 1350 million.

In the post green revolution period, waterlogging problem is a major concern affecting the agriculture production. In several places, waterlogging is a temporary phenomenon and it may or may not be harmful depending upon its severity and the nature of the agricultural practices followed at the time of waterlogging. It is caused by indiscriminate application of irrigation, lack of adequate drainage facilities, improper management of irrigation water sources and natural calamities like cyclones, floods etc. The estimate of waterlogged areas in the country varies with the source. In India, it has been estimated that 85.26 lakh ha. area is under waterlogging and particularly in Tamil Nadu the waterlogging area is around 0.18 lakh ha. (Suraj Bhan

et al., 2000). But the flood affected crop area for the period from 1953 to 1997 was estimated that of about 3.48 million ha. (Anonymous, 1999).

If rain or flooding continues for long periods, ponding of water takes place on the soil surface due to poor surface drainage. This ponding of water preventing the carbondioxide and other gases formed by plant roots and other organisms from being exchanged with oxygen of the atmosphere. In the absence of aeration (anoxia), root development and uptake of water and nutrients of most plants are reduced. Further, the plant root ecology, physical soil conditions, diseases and insect pests seriously affect the plant growth and hence the crop yields (Mamoria and Tripathi, 1991).

Generally pulses are very susceptible to damage by waterlogging under flooded condition. It varies widely in their sensitivity to injury in anaerobic soils, and within the same species the effect may vary depending on its stage of growth and on the environmental conditions. In sensitive plants, symptoms of injury can become evident in both roots and shoots and nodules (Russel *et al.*, 1990). Legumes may be particularly sensitive to flooding stress because they are dependent on N₂ fixation, a process that requires large amounts of O₂ which is deficient under waterlogging (Bacanamwo and Purcell, 1999).

The response to flooding stress in several species is associated with a number of biochemical, morphological and anatomical changes in both the root and shoot (Ricard *et al.*, 1994; Vartapetian and Jackson, 1997). Tolerance of water logging appears to be associated with the capacity to develop oxygen-conducting aerenchyma within roots and with a rate of endogenous ethylene production too low to inhibit root extension. Similarly the capacity of pulse nodules to survive and fix nitrogen in waterlogged soil is associated with the ability to develop loose structure that facilitates gaseous exchange. Nitrogenase activity is especially sensitive to ethylene and the rate of endogenous ethylene production under oxygen deficient conditions may be an

important factor in determining the sensitivity of legumes to water logging. More research in this area is needed to screen varieties for tolerance to waterlogging.

In flood-tolerant plants, the formation of aerenchyma and adventitious roots in the vicinity of cotyledonary nodes is an indicator of the presence of adaptive mechanisms (Kawase, 1981) to flooding stress.

In addition to the morphological and anatomical adaptations, the metabolic adaptations are critical for plant survival in anaerobic environments. The mechanisms involved are complex and include avoidance of accumulation of toxic compounds and maintenance of a continuous supply of energy. The proteins that are synthesized as a specific response to anaerobiosis are called the anaerobic polypeptides (ANPs). Several ANPs have been characterized by means of protein chemistry and the molecular approach. All the characterized polypeptides are glycolytic enzymes (Liao and Lin, 2001).

Alcohol dehydrogenase (ADH) was taken as a selection criterion as in absence of oxygen it recycles NAD^+ for continued glycolysis in the absence of oxygen. Rapid increase in ADH is considered as a powerful adaptive mechanism for waterlogging tolerance (Cao and Cai, 1991).

The role of plant growth regulators in imparting adaptation to flooding has been reviewed in detail elsewhere (Jackson and pearce 1991; Voesenek *et al.*, 1989). 6-Benzyl amino purine (BAP) alleviated more effectively the waterlogging effects by inducing closure of abaxial stomata in cotton plants (Goswami, 1990).

Relatively very few research studies dealing with the effects of waterlogging on plant growth and of the mechanisms involved have used pulses as experimental material. Very limited research available on the alleviation of waterlogging damage by applying a suitable plant growth regulator at appropriate growth stages. Based on

these backgrounds, the current study was conducted to determine the physiological and anatomical acclimation mechanism to flooding stress in pulses with specific objectives:

- i) to screen the genotypes of pulses for flooding tolerance
- ii) to understand the physiological, biochemical and molecular mechanisms of flooding tolerance in pulses
- iii) chemical manipulation for induction of flooding tolerance with chemicals and plant growth regulators.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Because of increased interest in wetlands and unpredictable excessive monsoon are a major concern in agriculture which increase the need to understand soil-plant relations in flooded soil (Kozłowski, 1984; Crawford, 1987; Kennedy *et al.*, 1992). All higher plants require water to be freely available for their establishment and survival, yet excess water can prove harmful or even lethal for food plants. The adverse effect of excess soil water (transient flooding or water logging) on the establishment and yield of many agricultural crops is well documented (Drew, 1992; Drew and Lynch, 1980). The extent of injury due to waterlogging depends upon the crops, genotypes, environmental conditions, stage of development and the duration of stress (Orchard and Jessop, 1984). The response to flooding stress in several species is associated with a number of biochemical, physiological, morphological and anatomical changes in both the root and the shoot (Ricard *et al.*, 1994; Vartapetian and Jackson, 1997).

The flooding effect is more severe for pulse crops particularly under shallow watertable condition. The literatures available on the effect of waterlogging on pulses are very limited. The contribution of early researchers on the response of waterlogging in different crops is reviewed in this chapter.

2.1. Screening methods for flooding tolerance

Nelson *et al.* (1983) developed three screening methods to determine flooding tolerance in 3 phaseolus bean genotypes empirically differing in tolerance. These methods consisted of (1) Triphenyl Tetrazolium Chloride reduction method (TTC), which measures flooding tolerance by determining the rate of root respiration during stress and indicate the status of oxidation reduction reaction in cell. Increasing ability to reduce TTC indicates increasing flooding tolerance. (2) Electrical Conductivity

method (EC), in which the degree of root cell membrane integrity is determined; and increasing amounts of cell contents leached indicating decreasing flooding tolerance. (3) Pressure Chamber method (PC), by which the degree of plant desiccation is found by measuring the water potential of the xylem. It can be expected that during flooding of plants, the tolerance to waterlogging will be directly related to the extent of TTC reduction ability maintained by the tissue.

Bin yan *et al.* (1996) studied the potted corn plants were flooded at the 4 leaf stage for 7-day flooding treatment in a controlled environment resulted in a significant decrease in membrane permeability due to the reduced activity of Superoxide Dismutase (SOD).

A pressure chamber technique (Scholander *et al.*, 1964) has been used intensively for rapid measurement of leaf water potential. Loss of membrane integrity following flooding stress, resulting in leakage of solutes, has been reported by Grineva (1962). A conductivity method (Dexter *et al.*, 1932) measuring ion efflux following stress injury has been used successfully to evaluate relative freezing and heat stress tolerance of potato species (Palta *et al.*, 1981). Reduction of 2-3-5-triphenyl tetrazolium chloride known as TTC method (Kittock and Law, 1968) has been used to determine relative heat and freezing stress resistance (Palta *et al.*, 1981).

Alcohol dehydrogenase activity was also taken as a selection criteria for screening genotypes to tolerant in the absence of oxygen, it helps by its oxidizing power. Rapid increase in alcohol dehydrogenase is considered as a powerful adaptive mechanism for water logging tolerance (Cao and Cai, 1991). In bitter melon, ADH activities were enhanced 9-fold over the control level after 3 days of flooding (Liao and Lin, 1995).

2.2. Morphological characters

2.2.1. Plant height

Height of the plant has been considered as an important morphological parameter related to growth and development of a plant. The cultivars with vigorous growth usually produce taller plants. These plants continue to grow vigorously until maturity and expected to yield more. Flooding modifies almost every aspects of shoot behaviour. The impact of waterlogging on shoot growth can be observed on changes in growth habit, visual health, internal anatomy, water relations, and hormonal and nutritional composition. Waterlogging can inhibit leaf and stem expansion and tiller production and cause epinastic curvature of petioles, orientation of shoot extension (Jackson and Drew, 1984). Sarkar *et al.* (1999) was observed that plant height after 12 days of submergence showed significant positive association with survival percentage.

Heatherly and Pringle (1991) showed that soil inundation of less than 48 h will result in the consistently significant plant height increase for soybean and that soybean genotype differ in their response to flood irrigation.

Sorte *et al.* (1997) observed that the better performance of soybean CP JS 80-21 under water logging may be attributed to its better plant height when averaged over three seasons than rest of the cultivars. Plant height was significantly reduced due to waterlogging and the maximum reduction (7.69%) was recorded in 8 days waterlogging at vegetative stage. The adverse effect of waterlogging was found at later growth period. Interaction between cultivars and stages indicated that height of cv MACS-58 was significantly less as compared to cv JS-80-21 at flowering stage. (Sorte *et al.* 1995 and Goswami, 1990).

Flooding decreased plant height significantly with increase in flooding duration at vegetative and flowering stages in mungbean. Flooding at vegetative growth stage for 3, 6 and 9 days duration decreased plant height by 81, 28 and 61

per cent, respectively while this decrease was upto 10, 23 and 53 per cent, respectively, when flooded for same period at flowering stage. This indicates that with advance in stage tolerance increases (Jhorar *et al.*, 1995).

Exposure to anaerobic condition for short periods eg., 24 h, can sometimes lead to permanent reduction in plant growth. A significant difference in plant height between flooded and non flooded treatments was found for both growth stages vegetative and flowering. However this difference appeared after the first week of the flood in the case of flowering and the second day of the flood at vegetative stage (Sallam and Scott, 1987).

2.2.2. Root parameters

Flooding suppresses the formation of root hairs and branching of roots. Also permeability of the root cells decreases due to the shortage of oxygen.

According to Keelay (1979) the waterlogging tolerant ecotype of *Nyssa sylvatica*, when waterlogged, partially loses its original roots while initiating new roots that are more succulent, larger in diameter, and less branched than non waterlogged roots. Under continued waterlogging of one year, such roots are replaced with a root system superficially resembling drained roots having increased internal O₂ transport. Root extension and root area was significantly reduced by 7 day after flooding at both vegetative and flowering growth stages. This reduction was due to suppression of root extension and to the killing and inhibition of root hair development (Sallam and Scott, 1987).

Suppression of root growth by flooding was also reflected in the dry weight of the root. Flooding at both vegetative and flowering growth stages significantly reduced the dry weight of soybean roots by 7 days. This reduction is attributable to suppression in growth of the main root, greater loss of roots, exudation and lower photoassimilate partitioning of flooded roots (Smucker, 1985).

Under poorly drained conditions, the rate of tap root and lateral root extension was significantly reduced, while the magnitude of response varied with cultivars (Store, 1985). Waterlogging reduced root volume by 18 to 39 per cent, the maximum reduction (39%) was obtained by 8 days waterlogging.

The length of living root was affected more adversely when flooded at 28 DAS as compared to when flooded 42 DAS. At both the stage of flooding, root length decreased with increase in flooding period. Flooding decreased root density at all depths, lower root density in whole profile as well as in individual layer might be due to prevailing anaerobic condition or reduced oxygen diffusion rate for longer period (12-15 days) (Jhorar *et al.*, 1995).

2.3. Growth attributes

2.3.1. Leaf area

Leaf area (LA) is an important photosynthetic parameters in crop plants which as shown positive relationship with net photosynthetic activity. LA has been shown to influence the radiant energy interception. LA is a major yield determining factor through dry matter accumulation (Watson, 1952).

The decrease in leaf area related to soil saturation suggests reductions in the source of photosynthates. Calculating the total number of roots produced per unit of leaf area was an attempt to explore the effect that these reductions had on the relative sink activity of saturated roots as compared with non-saturated root. Sink strength is the integration of sink size and sink activity. Root numbers indirectly indicate the size of the sink. The ratio of total root length to total leaf area provides an approximation to evaluate sink activity, defined as the capacity to use photosynthates (Lizaso and Ritchie, 1997).

The water logging at anthesis in sunflower severely reduced the both leaf area (with concurrent increases in senescent leaf number) with relatively little effect on

sorghum. Variation between species and stages of development may arise as a result of differences in growth and developmental process, which occur at the times of waterlogging. In contrast, waterlogging at initiation and the buds-visible stage resulted not only in reduced leaf expansion, but also in reduced stem extension and increased leaf senescence (Orchard and Jessop, 1984).

Waterlogging reduced leaf area significantly at all stages of soybean causing 6.5 and 13.9 per cent reduction by 4 days and 8 days waterlogging (Sorte *et al.*, 1995). The Sugimoto *et al.* (1989) also recorded that leaf area extension was markedly suppressed by waterlogging during flower bud differentiation.

2.3.2. Specific Leaf Weight (SLW) and Specific Leaf Area (SLA)

Greater understanding of how waterlogging reduces yield would be gained by identifying how growth dynamic parameters such as specific leaf weight, specific leaf area and leaf expansion rate (LER) are linked with yield components associated with the yield loss.

Specific leaf area (SLA) is a measure of leaf thickness which varies with cultivar, leaf position, growth stage and environment. The SLA increased markedly during the early part of the vegetative phase. Usha Rani (1979) noted that SLA was inversely related with SLW. Specific leaf weight (SLW) is the leaf dry weight per unit leaf area, is also an useful morphological character can be used as a selection criterion while evaluating the yield potential of crop plants (Dornhoff and Shibles, 1970).

Dias-filho *et al.* (2000) observed that flooding reduced specific leaf area in all *Brachiaria* spp. The SLA was corroborative to the LAR (Singh and Pandey, 1997). Wei-fengzhen *et al.* (2000) recorded that the leaf area and weight and specific leaf weight all decreased at grain formation and filling stages of wheat.

Haloi (1989) marked that SLW at 50 per cent flowering stage showed direct bearing on productivity. A highly significant positive relationship between SLW and grain yield was recorded evidently suggesting that SLW might have enhanced photosynthesis after flowering in deepwater rices under keep water stress situations leading to higher grain yield.

2.4. Drymatter production

The first prerequisite for high yields is an increase in the total dry matter per unit area. Carbon compounds account for 80-90 per cent of the total dry matter produced by the plants. It is quite evident that photosynthetic process is the basis for the building up of organic substances and ultimate dry matter production (Adak and Das Gupta, 1999).

Laan *et al.* (1991) reported that flood tolerant *Rumex maritimus* and *Rumex crispus* had more biomass production. But the flood-intolerant *Rumex thyrsiflorus*, however, only few slow-growing new roots were developed and biomass production was significantly reduced at solution oxygen concentration below 2% (V:V).

Soil water saturation caused a reduction in final biomass (Meyer *et al.*, 1987), with a higher reduction when saturation occurred earlier in the season (Mukhtar *et al.*, 1990).

Plant biomass in the saturation treatments was expected to decrease, since leaf area and photosynthesis were reduced; however, above ground biomass was about the same for saturated and nonsaturated maize plants during the saturation period. Reduced photosynthesis after lowering of the water table to end saturation caused a reduction in biomass by harvest date in the range of 40 to 50% in saturated treatments as compared with the control (Lizaso and Ritchie, 1997).

Dry matter is an important criterion as it determines the source-sink relationship. Dry matter reduction has ranged from 12 to 22 and 17 to 25 per cent due to 4 days and 8 days waterlogging during all stages respectively. Severe reduction (25%) was found during flowering stage (50 DAS) (Sorte *et al.*, 1996).

Yadav and Saxena (1998) reported that under nine days of waterlogging, mungbean variety T44 had maximum accumulation of dry matter per plant followed by susceptible genotypes Pant M2 and Pant M1. Better adaptability and stability in variety T44 under higher waterlogging stress appears to be bestowed by higher accumulation of dry matter in plant.

2.5. Nodulation status of pulses

2.5.1. Nodule number and Nodule dryweight

The efficiency of symbiotic nitrogen fixation in legumes is positively correlated with nodule number present in root and nodule occupancy (Weber, 1966a). For many legumes, nitrogen accumulation is slowed by waterlogging, due in part to reduced nodulation and the decreased specific activity of nitrogenase (Minchin and Pate, 1975).

Cowie *et al.* (1996) observed that control chickpea plants had large multilobed nodules, which were generally green with pink tips, while plants treated with ethephon had many small pink nodules, and no nodules were present on waterlogged plants.

Flooding at preflowering growth stage completely inhibited nodulation in soybean roots. Rhizobia are strictly aerobic and the nodules have a high oxygen uptake which is two to three times that of soybean roots. There is no doubt that this high oxygen demand of nodules was not met under flooding conditions (Sallam and Scott, 1987). Both Nathanson *et al.* (1984) and Troedson *et al.* (1989) found that after

the initial acclimation to a saturated soil, soybean nodule weight increased significantly over that in conventionally irrigated soybean.

For the formation and functioning of nodules a liberal supply of oxygen in the root environment is essential and even a thin layer of water on the nodule surface, suppress nitrogen fixation (Sprent, 1971).

Nodule mass showed a highly positive correlation with symbiotic nitrogen fixation (Weber, 1966b). Mean total nodule dry weight per plant decreased markedly with increasing periods of waterlogging upto 8 duration in cowpea but remained fairly constant thereafter minimum values (0.26 g) were recorded for plants which experienced 16 days waterlogging although those stressed for 32 days had slightly greater total nodule dry weight (0.33 g) (Minchin and Summerfield, 1976).

Pugh *et al.* (1995) reported that the infected cells of submerged nodules were larger and had larger vacuoles than those from nodules of normally watered plants. It is suggested that this increase in vacuoles volume to protoplast volume in infected cells may play a role in the tolerance of white clover nodules to waterlogging.

There are several reports suggesting that nodulated grain legumes growing in waterlogged conditions generally fix less nitrogen and produce less dry matter and total nitrogen than in waterlogged conditions. This phenomenon has been considered a physiological one since oxygen transport to and within the nodule is impaired (Walker *et al.*, 1983).

Inhibition of nodulation of waterlogging has been reported in several legume species (Huang and LaRue, 1985). The capacity of root nodules of legumes to survive and fix nitrogen in waterlogged soil is associated with the ability to develop a loose structure that facilitates gas exchange (Smith, 1984).

Both nodule numbers and weights build up rapidly after anthesis, but evidently deteriorated after commencement of seed fill. Nodule number and nodule weights were reduced by waterlogging at both growth stages. The anthesis waterlogged plants resumed nodulation after being removed from the 10 days waterlogging, but at a slower rate than the control (Sung, 1993). Sallam and Scott (1987) suggested that the reduction of nodulation was attributable to the suppression of root growth and root hair development. In addition, owing to anaerobic condition, energy supply for the formation of nodules may be reduced (Kozlowski and Pallardy, 1984).

2.5.2. Nitrogenase

The assessment of biological nitrogen fixation and nitrogenase activity has been aided in recent years by employing the acetylene reduction technique (Dilworthy 1966). Andreeva *et al.* (1987) reported that waterlogging reduces nitrogenase activity and irreversibly alters ultrastructure of the cell in soybean root nodules.

Sung (1993) shows that water logging at both anthesis and commencement of seedfilling strongly influences nodule nitrogenase activity and leaf nitrate reductase activity of soybean plants. Waterlogging at anthesis significantly inhibited nitrogenase activity but the reduced activities could be resumed following the release of 10 days stress.

The nitrogenase activity of field-grown *Vicia faba* plants was measured by the acetylene reduction method and it was found that activity declined significantly when water content exceeded field capacity and led to depressed aeration, as well as when the soil dried below field capacity (Sprent, 1972).

Day *et al.* (1975) indicated that endogenous ethylene can inhibit the nitrogenase activity of subterranean clover (*Trifolium subterraneum* L.). N_2 -fixation activity of soybean expressed as mmole C_2H_2 /plant/hour were by far greater in the soil

saturated conditions than the conventional irrigation in the dry season (Pookpakdi, 1993; Hunt and Hayzell, 1993).

2.5.3. Leghaemoglobin

In the root nodules of leguminous plants, a variable diffusion barrier restricts the oxygen concentration in the cells, but facilitation of its diffusion by leghaemoglobin and the high affinity for oxygen of the bacteroid cytochrome-C oxidase allow the metabolism of both the infected cell and the bacteria to be aerobic under a wide range of external oxygen concentration (Vance and Heichel, 1991).

The anaerobic environment probably has led to the arrest of nodule bacteroid respiration and metabolic activity which eventually resulted in the depression of leghaemoglobin (Lb) contents because the heme part required for the synthesis of Lb must be provided by the microsymbiont (Nadler and Avissar, 1977). The increase observed in the content of some of the hemoprotein bands possibly indicate that the nodules synthesised them in excess to counter the negative effects of flooding (Selvaraj and Karunambal, 1995).

The function of leghaemoglobin was generally accepted as the facilitation of oxygen flux to the vigorously respiring *Rhizobium* bacterioids (Elfolk, 1972 and Appleby, 1998). It is well documented (Appleby, 1974) that leghaemoglobin content of most legumes root nodules is correlated with their nitrogen fixing ability.

2.6. Physiological changes

2.6.1. Leaf Gas Exchange parameters

Leaf gas exchange parameters have been used to study the photosynthetic capacity of plants during flooding. Except in some flood-tolerant plants that have developed adaptive mechanisms so as to maintain better photosynthetic capacity (Topa and Cheeseman, 1992), flooding causes a significant decrease in the capacity for leaf gas exchange in most flooding-intolerant plants. Examples are *Triticum*

aestivum (Trought and Drew, 1980a), *Lycopersicon esculentum* (Bradford, 1983), *Pisum sativum* (Jackson and Kowaleska, 1983), *Momardica charantia* (Liao and Lin, 1994). A decrease of stomatal conductance was found to be correlated with an increase of leaf water potential in flooded bitter melon (Liao and Lin, 1994). In control and flooded plants, stomatal conductance were shown to be correlated with CER (CO₂ Exchange Rate) (Vu and Yelenosky, 1991). The internal CO concentration (C_i) of leaves was found to increase linearly with the duration of flooding, despite a reduction of stomatal conductance (Liao and Lin, 1994 and 1996).

Waterlogging undoubtedly depresses net carbon fixation per unit of leaf area when calculated as a net assimilation rate (Trought and Drew, 1980a). Bradford and Hsiao (1982) reported that inhibition of ribulose biphosphate carboxylase/oxygenase production is partly responsible, together with stomatal closure. A decrease of CER, independent of stomatal conductance, in sunflower was however reported by Guy and Wample (1984).

Flooding has been reported to cause stomatal closure directly, without affecting the photosynthetic capacity in *Vaccinium astei* (Davies and Flore, 1986a, 1986b). Liao and Lin (2001) suggested that stomatal aperture is not the only limiting factor for CER but is partly responsible for the decrease in the photosynthetic capacity of mesophyll tissue. Also feed back inhibition of starch accumulation may result in a reduction of CER in flooded plants.

Flooding also closed stomata of *Betula papyrifera* seedlings for at least 14 days. Observations were discontinued after 14 days because leaves of flooded plants were yellow and stomata had lost their regulatory function (Tang and Kozlowski, 1982c). In seedlings of the flood tolerant species *Fraxinus pennsylvanica*, stomata began to close within a day or two after flooding was initiated, and they continued to close progressively for the next 7 days. Stomata of flooded seedlings began to reopen after 15 days of flooding. Stomatal reopening was closely correlated

with production and growth of adventitious roots on submerged portions of stems (Kozlowski and Pallardy, 1979).

Plants that are readily injured by flooding also show a rapid reduction in the rate of photosynthesis (P_n). Stomatal closure appeared to be mainly responsible for the rapid decrease of P_n (Phung and Knipling, 1976).

P_n of flooded plants appeared to be reduced first primarily because of stomatal closure and later by a reduction in photosynthetic capacity as well. Hence, in the longer term reduction in photosynthesis is partly traceable to changes in carboxylation enzymes, reduced chlorophyll content of leaves, early leaf senescence and abscission and reduced leaf area (Kozlowski, 1982).

Bradford (1982) found that stomatal closure was not the only factor limiting photosynthesis of flooded *Lycopersicon esculentum* plants. Bradford concluded that a combination of ABA accumulation and decreasing cytokinin supply might induce stomatal closure. The decrease in cytokinins also resulted in lowered ribulose biphosphate regeneration capacity. The end result was a reduction in P_n , without reduction in bulk leaf ψ . Stomatal closure is caused in leaves of flooded plants by ABA which accumulates soon after flooding, prior to any leaf dehydration and may occur at high relative humidities (Jackson, 1991).

Baruch (1994) reported that in *Andropogon gayanus* and *Hyparrhenia rufa* showed early stomatal closure without concurrent increase in ψ and leaf turgor. This low stomatal conductance which caused reduced P_n . There was a slight increase of stomatal conductance in the middle of the flooding period and both grasses recovered g_s and P_n after drainage.

Stomatal conductance and net carbon assimilation were reduced significantly in response to hypoxia by 46 and 18% in *Spartina patens*, respectively (Pezeshki

et al., 1993). One of the first responses of photosynthesis to root zone flooding is stomatal closure in sensitive species. This phenomenon has been observed in a number of different species, including important crops such as tomato, wheat, pepper and bean (Pezeshki, 1994).

Jiang and Lenz (1995) reported that the increased transpiration during short term waterlogging were due to wide opening of the stomata. After 10 days, the stomata were closed and the leaves wilted; dark respiration exceeded net P_n .

2.6.2. Leaf water potential

Leaf water potential (LWP) was considered as a good indicator of plant water status since it was associated well with different plant functions and productivity in legumes (Sharratt *et al.*, 1983; Phogat *et al.*, 1984). Water relationships of waterlogged plants have been studied extensively in both woody (Kozlowski and Pallardy, 1984) and herbaceous plants (Jackson and Drew, 1984).

Perez *et al.* (1999) studied that decrease of leaf water potential in tomato seedlings at the beginning of flowering for 72 h flooding. Plants of castor bean were waterlogged for 15 days decreased the leaf osmotic potential (Gadallah, 1995). Bishnoi and Krishnamoorthy, (1992) observed that waterlogged groundnut cv. MH-2 had slightly decreased the leaf water potential (ψ leaf).

Flooding during the vegetative and reproductive growth stages did not significantly affect the components of leaf water potential for either the Essex or Forest cultivars of soybean. The water potential of control and flooded plants varied between -0.62 and -0.92 MPa during the week that the flood was imposed. Components of leaf water potential were not affected by flooding in soybean, indicating that reductions in P_n and g_s were not directly associated with leaf water-deficits. Fourteen days after the flood was removal, P_n and g_s continued to be depressed in soybean cultivars (Oosterhuis, *et al.*, 1990).

Shoot responses to root hypoxia have been reported to be mediated both by changes in leaf water status and by ABA transported from the roots (Zhang and Davies, 1987). Neuman and Smit (1991) attempted to determine the relative roles of leaf water status and root-sourced ABA in mediating the stomatal and leaf growth responses of *phaseolus vulgaris* seedlings exposed to rapid depletion of root zone oxygen. When plants under high Vapour Pressure Deficit (VPD) were exposed to root hypoxia, there was a temporary decrease in leaf water potential. The timing of the changes in leaf water potential correlated with the initial reduction in stomatal closure. In rapidly, transpiring bean plants, therefore, the initial changes in stomatal conductance can be accounted for by decreasing leaf turgor. Waterlogging reduced the total photosynthetic area and the rate of photosynthesis in sorghum (Bhagwat *et al.*, 1986).

According to Sharma and Singh (1994), the subdued rate of photosynthesis might be attributed to the combination of reduced light, oxygen and chlorophyll attenuation under submergence.

Stomatal conductance is reduced in tomato leaves following waterlogging, without the intervention of a transient wilting and recovery cycle. Moreover, stomatal closure is one of the earliest shoot responses to waterlogging, coinciding with the development of epinasty (Bradford and Hsiao, 1982). Stomatal conductance and transpiration are reduced by 30% to 40% after approximately 24 hrs of soil flooding. This is not due to a transient water deficit, as leaf water potential is unchanged.

Singh *et al.* (1991) studied that flooding affects photosynthesis before stomatal conductance or leaf water potential in snap bean. However, as duration of flooding increases, photosynthesis becomes closely linked with decline in stomatal conductance. The plants recover slowly after flooding stress removal; Ψ and gas exchanges of stressed plants remain significantly lower than the control even after recovering for 7 days.

2.6.3. Chlorophyll fluorescence

Fluorescence is an analytically useful emission spectroscopic technique in which atoms or molecules are excited by the absorption of electromagnetic radiation, the excited species then relax to the ground state, dissipating their excess energy as photons (light). During the photosynthetic processes of light absorption and energy transduction, a small percentage of the absorbed light, not utilized in photochemistry, is re-emitted as fluorescence. Chlorophyll fluorescence has been dubbed as the plant physiologist stethoscope because they are extremely useful in the diagnosis of photosynthetic activity of plants under normal and stressed conditions (Krause and Weis, 1991).

The difference between maximum fluorescence signal (F_m) and the low level (F_o) is said to be variable component of fluorescence. Ratio of F_v/F_m is directly proportional to the quantum yield of photochemistry and show high degree of correlation to quantum yield of net photosynthesis of intact leaves (Krause and Weis, 1984).

According to Sharma and Singh (1994), the subdued rate of P_n might be attributed to the combination of reduced light, oxygen and chlorophyll attenuation under submergence. However, the tolerant varieties were able to survive due to slow reduction of chlorophyll fluorescence in leaves owing to their elongation ability above the water level.

Adams and Perkins, (1994) indicated that Chlorophyll fluorescence is a rapid, consistent and reproducible method of determining low temperature tolerance to spruce foliage. Chlorophyll fluorescence induction kinetics has been widely used in studies to determine how a range of environmental factors, alter the photosynthetic capacity of plants. The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) describes the photochemical efficiency of photosystem II (PS II), also ratio correlates very well with the Quantum yield of net photosynthesis.

Wullschelger *et al.* (1992) indicated that the efficiency by which excitation energy was captured in PS II (Fm-Fo/Fm) was reduced from 0.80 to 0.73 after 4 h exposure of soybean leaves to 10 $\mu\text{l/l}$ ethylene. This reduction was also accompanied by a 12% decrease in steady state photochemical quenching (qp), indicating that a lower proportion of open or oxidized PS II reaction center were participating in light dependent process. Thus photoinhibition may play a role in the ethylene-induced inhibition of CO₂ assimilation. Fv/Fm can be used on probe to monitor the activity of photosynthetic carbon assimilation with field.

2.7. Biochemical Mechanisms of flooding tolerance

The tolerance of oxygen deficiency differs among various species ranging from hours to months. In species tolerant of flooding, flood tolerance is achieved through various means including anatomical and biochemical adaptation.

2.7.1. Soluble proteins and root anaerobic proteins

The root and leaf protein contents in maize seedlings showed a decreased in the soluble protein level under all treatments during waterlogging. However, the 30-day-old seedlings exhibit more prominent decrease in protein as compared to 15-day old seedlings. Sinha *et al.* (1995) and Sachs *et al.* (1980) have observed a repression of the normal protein synthesis during anaerobiosis but not a complete inhibition of the gene expression, although complete dissociation of polysomes have also been observed. Das *et al.* (2000) found that a negative correlation between the duration of waterlogging and titre of some primary metabolites like total soluble protein and RNA. Flooding for 4 days in lupin and transgenic tobacco plants have decreased concentration of soluble protein (Yu Qin and Rengal, 1999).

Plants also respond to anoxia by altering the pattern of root protein synthesis. The proteins which are synthesized as a specific response to anaerobiosis are called the anaerobic polypeptides (ANPs) (Sachs *et al.*, 1980). Protein chemistry and the molecular approach have characterized several ANPs. All the characterized

polypeptides are glycolytic enzymes (Mujer *et al.*, 1993). Among the ANPs, ADH is predominating one and has been extensively studied (Sachs *et al.*, 1980). New synthesized ADH isozymes emerge during flooding in many plants (Lin and Lin, 1992; Liao and Lin, 1995) and with different biochemical properties. The reaction catalyzed by the enzyme is, however, not the rate-limiting step in ethanolic fermentation, and the regulatory significance of the mass induction of ADH remains unclear (Bertaini and Reggiani, 1991).

The anaerobic root proteins were first described and identified in the maize root (Sachs *et al.*, 1980; reviewed by Kelly, 1989), and are more recently identified in rice embryos (Ricard *et al.*, 1994) and seeds (Perata *et al.*, 1992) as well as in soybean roots (Russell *et al.*, 1990). They were all involved in the pathways, which mobilize sucrose or starch for ethanol fermentation, i.e., that necessary to maintain energy production under anaerobic conditions.

The type and extent of ANP synthesis is directly related to the severity of oxygen deprivation, and to the plant species. In wheat, synthesis of ANP was optimal at hypoxic concentrations of oxygen and did not occur under anoxia, whereas in rice, ANPs were not induced until lower threshold oxygen concentrations and continued even under anoxia (Bertani and Brambilla, 1982). In maize roots, more complex patterns of proteins are synthesized under hypoxia than anoxia; some proteins are glycolytic enzymes, whereas others are hypoxia specific responses (Bennett and Freeling, 1987). Oxygen deficiency and ANPs in maize have been used as a model system to study gene regulation because during hypoxia regulation occurs at both the transcriptional and translational levels, and involves both positive and negative factors (Drew, 1990).

The few long-term studies on ANPs have used in rice or *Echinochloa* (barnyard grass) because these species can germinate and grow for several weeks in an anaerobic environment. Expression of ANPs is similar among flood-tolerant and

flood-intolerant species of *Echinochloa*; however, induction of ANPs is much faster in intolerant species (Mujer *et al.*, 1993).

In the waterlogging sensitive species, *Brassica napus*, diminution in total free aminoacids and soluble proteins was more pronounced, whereas the tolerant species, *Brassica juncea* showed a marked increase in soluble proteins and the moderately tolerant, *Brassica carinata* in the aminoacids. Both have played same role in sustaining growth under waterlogged condition (Ashraf and Mehmood, 1990).

2.7.2. Chlorophyll Pigments

The role of chlorophyll (chl) in photosynthesis has been well established (Sestak, 1971). On flooding, all terrestrial plants become chlorotic within 3-4 days. Four to five days of soil waterlogging gives a noticeable loss of chlorophyll in the oldest leaves of barley plants (Leysdon and Sheard, 1974). The importance of chlorophyll content to photosynthesis is emphasized by decline in both chlorophyll and photosynthesis in mineral deficient plants. During the period of submergence however, there was a reduction of chlorophyll content both in tolerant and susceptible cultivars. Reduction rate was higher in CR 383-10 and IR 42 as compared to tolerant rice cultivar FR 13A (Das and Sarkar, 2001). The overall decrease in chlorophyll contents during different periods of waterlogging has also been reported by Krishnamoorthy *et al.* (1981) in peanuts. Sinha *et al.* (1995) observed that the shift in chlorophyll a/b ratio could be taken as an indicator of plant adaptation during anaerobiosis for their maximum energy production as well on for the generation of reducing equivalents, which could be later utilized for the biosynthesis of carbohydrate by reductive pentose phosphate cycle. Corn plants flooded for 7 days showed accelerated senescence of the bottom leaves to the upper leaves, which was reflected by the loss of chlorophyll content to plants grown in a well drained conditions (Bin Yan *et al.*, 1996).

2.7.3. Carbohydrate fractions

Several studies showed that the starch levels in intact roots of alfalfa and rice did not significantly vary under increased duration of flooding, suggesting that root starch is not readily mobilized and converted to fermentable substrates (Barta, 1988; Bertani *et al.*, 1981). However, Perata *et al.* (1992) reported that germinating rice seeds could degrade starch reserves under anoxia, whereas wheat seeds did not germinate and were unable to degrade the starch in the endosperm. These distinct behaviours are due to the successful induction of α -amylase under anoxia in rice seeds but not in wheat seeds.

Accumulation of starch has been reported in leaves of various flooded plants, e.g., *Helianthus annuus* (Wample and Davis, 1983), *Citrus sinensis* grafted onto rough lemon and sour orange root stocks (Vu and Yelenosky, 1991) luffa (Su *et al.*, 1998) and wax-apple tree (Hsu *et al.*, 1999). Under root hypoxia, starch accumulation in leaves has been attributed to a reduced rate of translocation of carbohydrates from leaves to roots (Barta, 1987) and to retarded growth and a lower level of metabolism in roots, which apparently causes the carbohydrate demand to decrease (Hsu *et al.*, 1999).

Barta (1988) reported that there was no significant pattern of change in root starch concentrations with increased duration of waterlogging, indicating that root starch probably is not being utilized to a greater extent than the control treatments. This result is similar to that observed in flooded rice (*Oryza sativa* L.) roots (Bertani *et al.*, 1981). Carbohydrate status before submergence played a vital role to withstand submergence stress (Sarkar, 1998).

Selection for the improvement of submergence tolerance, therefore can be useful if it is based on nonstructural carbohydrates (sugar + starch), NSC content/seedling measured before inundation (NSC) (Sarkar *et al.*, 1999).

2.7.4. Nitrate Reductase Activity (NRase)

NRase is the first enzyme to reduce nitrate to nitrite and activity was equal or higher in adventitious roots than in seminal roots of corn plants. Tsai and Chu (1993) suggest that increasing activity of nitrate reductase in corn roots under waterlogged conditions probably promotes nitrate uptake, and the nitrate may play a special role during oxygen deficiency by acting as an alternative electron acceptor to oxygen in corn roots, as well as helping to eliminate excess NADH by sufficient oxygen availability (Lambers and Smakasam, 1978).

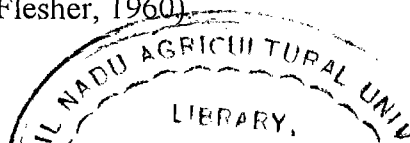
The marked reduction in NRase during submergence indicated a change in metabolic activity especially protein metabolism in the leaves. NRase in the roots of plants after submergence was negatively correlated to submergence tolerance. After submergence, plants with low root NRase reduced nitrate slowly and the immediate demand for carbohydrate in the roots decreased (Mazaredo and Vegara, 1981).

Nitrate reductase is thought to play a key role in the regulation of assimilatory nitrate reduction [Sung and Sun, 1990; Hoff et al., 1992]. Reduction of NRase in leaves of waterlogged plants in the rapid depletion of the nitrate as oxygen is consumed by soil biota as anaerobic conditions develop. As a result, volatilization and loss of nitrate are promoted through denitrification (Kozłowski and Pallardy, 1984). The activity of NRase is decreased in flooded leaves of wheat (Youngping *et al.*, 2000). Fewer nitrates taken up by the waterlogged leaves in comparison with the controls as a result of low oxygen effect on root metabolism (Van Toai and Bolles, 1991).

2.8. Ethanol Fermentation Enzymes

2.8.1. Alcohol Dehydrogenase (ADH) and Pyruvate Decarboxylase (PDC) Enzymes

Plant responds to anaerobic conditions by altering the expression of various genes. Studies have found that ADH activity increases during anaerobic stress in many plant species including maize (Hageman and Flesher, 1960).



ADH is considered as essential for anoxia survival, presumably because it recycles NAD^+ for continued glycolysis in the absence of oxygen (Russel *et al.*, 1990; Kennedy *et al.*, 1992). It is only at the very low levels of ADH activity found in ADH 1 Null mutants that enzyme activity restricts fermentation, energy metabolism, and anoxia tolerance (Johnson *et al.*, 1994).

Alcohol Dehydrogenase (ADH) is responsible for the synthesis of alcohol and regeneration of NAD in alcoholic fermentation. This regenerated NAD enables glycolysis to continue under anoxia, thus producing a net 2 moles of ATP per mole of glucose relative to the 38 moles of ATP produced under aerobic conditions through respiration (Davies, 1980).

When four varieties of 10 day old rice seedlings were submerged for 3 days there were 4-5 fold differences in activities of ADH, whereas there were no major differences in the activities of non submerged plants. The greatest increase in ADH activity occurred in the most tolerant variety (IR 42) and a cross of these two varieties (IR 26702-25) had intermediate submergence tolerance with intermediate increase in ADH activity (Setter, 1993). These results are consistent with published data for barley and rice (Wignarajah *et al.*, 1976), which showed that flooding tolerance was associated with increased ADH activity.

For most plants it more likely that Pyruvate Decarboxylase (PDC) is the rate limiting enzyme in alcoholic fermentation since it controls the entry of carbon into the pathway (Setter, 1993). Waters *et al.* (1991) presented convincing evidence that PDC is rate limiting alcoholic fermentation in wheat exposed to anoxia.

The inducible formation of ADH that accompanies ethanol production has been studied in the course of characterizing flood-tolerant and intolerant plants (Crawford, 1967; McManmoh and Crawford, 1971). High levels of ADH activity and ethanol production during anaerobiosis have been reported for flood-tolerant plants

(Tripepi and Mitchell, 1984). The activity of ADH was positively correlated with the magnitude of flood injury in different genotypes (Liao and Lin, 1995; Lin and Lin, 1992) and species with higher ethanol production had less tolerant to flooding, compared to tolerant plant species (Barta, 1984).

The level of ethanol production in flooded sunflower and luffa roots does not continue to increase in proportion to the levels of PDC and ADH activities but declines after an initial rise on day one (Jayasekaran *et al.*, 1990; Su and Lin, 1996).

Chrissmall *et al.* (1993) concluded that the induction of ADH in axes of N₂ incubated seeds may be casually related to the ability of *Erythrina caffra* seeds to germinate anaerobically. If ethanolic fermentation is necessary for anoxic germination of *Erythrina caffra* this should be reflected in the induction of ADH in embryonic axes.

Activities of alcohol dehydrogenase (ADH) and endogenous concentrations of ethanol increased gradually, reaching a maximum level on the third day of flooding in *Momardica charantia* (Liao and Lin, 1995).

Pandey (2000) reported that effect of different duration of waterlogging (hypoxic conditions) increases the root NAD-alcohol dehydrogenase (ADH) activity from the control samples in zea mays; while during the post-hypoxic conditions the root ADH shows a decreasing pattern and attained value of control plants in 96 hour.

Notably, the activity of PDC is usually many-fold lower than that of ADH, suggesting that the rate-limiting enzyme for ethanol synthesis in flooded roots is more likely to be PDC than ADH (Su and Lin, 1996). Waters *et al.* (1991) suggested that a high ratio of ADH/PDC may be required to prevent accumulation of potentially toxic acetaldehyde.

The enhanced ADH activity and ethylene production are found in plants subjected to hypoxia support the postulate that these metabolites have adaptive significance for plants under hypoxic conditions (Pezeshki *et al.*, 1993; Mohanty *et al.*, 1993).

ADH activity showed some correlation with survival and PDC activity was correlated with ethanol fermentation, though *in vitro* activity of both enzymes was higher than the estimate rate of ethanol fermentation (Ellis and Setter, 1999).

Exposing plants to conditions which induce the response (low but not anoxic oxygen concentrations) greatly improves anoxia stress tolerance in a number of plants like wheat seedlings (Waters *et al.*, 1991) and maize roots (Johnson *et al.*, 1989).

Flooding induced an increase in activity of both PDC and ADH enzymes. While ADH activity was more affected by flooding temperature and seed quality than was PDC activity, no consistent correlation between ADH activity and flooding susceptibility was observed across genotype, flooding temperature and seed quality (Van Toai *et al.*, 1985).

Ethanol production, like transpiration, is sometimes considered a necessary evil. The metabolism of ethanol and its secretion have also been studied and further it has been proposed that malate, lactate, shikimic acid, and glycerol might be alternative metabolites which would avoid the toxicity of ethanol but still result in the regeneration of NAD (Torres and Diedenhofen, 1981). Saglio *et al.* (1988) reported that hypoxic training in maize root tips, which confers an improved energy status and tolerance of anaerobic conditions is closely linked to induction of an effective ethanolic fermentation pathway. Acclimation of root tips of maize seedlings by low O₂ before anoxia appeared to be necessary for full induction of ADH (Andrews *et al.*, 1994).

Chang *et al.* (1983) suggested that the rate-limiting enzyme for ethanol biosynthesis in sweet potato storage roots under anoxia is likely to be the pyruvate decarboxylase rather than alcohol dehydrogenase. Chang *et al.* (1982) indicated that the apparent activities of PDC, ADH, and ethanol concentration in sweet potato roots were higher in submerged roots than in aerobic roots.

ADH shows a minimal induction during flooding of alfalfa roots, whereas ADH activity is enhanced over four fold in birds foot trefoil (Barta, 1984).

In anoxia-tolerant *schoenoplectus lacustris* and certain other rhizomatous species as well as in beet root storage tissue, it is evident that glycolysis followed by fermentation to ethanol is the major pathway of respiration (Zhang and Greenway, 1994).

2.9. Malate Dehydrogenase - Alternative fermentation pathway enzyme

One metabolic adaptation that plants could use to avoid the production of ethanol by the glycolytic pathway is to use an alternative glycolytic pathway with an end product other than ethanol. Plant can utilize several different fermentation pathways that reduce NADH to NAD⁺. The end products could be lactate, malate, succinate or ethanol (Nilson and Orcutt, 1996).

Malate failed to accumulate under anaerobic conditions (4hr), and appreciable activity of malic enzyme was found in the roots of these flood tolerant plants (Davies *et al.*, 1974).

In flood-tolerant species there was evidence that phosphoenolpyruvate (PEP) was converted to oxaloacetate by fixation of CO₂ through the action of PEP carboxylase. Malate dehydrogenase then reduced oxaloacetate to malate, thus allowing the regeneration of NAD, to maintain glycolysis. Malate was assumed to be less toxic than ethanol. It was successful that any further conversion of malate to

pyruvate or to ethanol was prevented in flood tolerant species either by constitutive lack of appropriate enzyme (NADP-malic enzyme) or inhibition of its actions (Crawford, 1967; McManmon and Crawford, 1971).

Malate produced in flooded luffa root is easily decarboxylated, and adaptation of luffa seedlings to flooding appears to be incompatible with RMM Crawford's theory (Su and Lin, 1996).

MDH levels increased seven fold in *Brassica mutica* roots and decreased to half in leaves during flooding and remained unchanged in *Brassica brizantha* (Sevaram, 2000).

2.10. Free radical scavenging enzymes

Oxygen radicals are normally produced in biological tissues as a consequence of oxidative metabolism and during electron transport. Under normal conditions these reactions are tightly controlled, but during stress, radical becomes cytotoxic and causes oxidative injury to cellular components. Conditions favouring oxygen radical generation, like low energy charge, high reducing equivalent levels and saturated electron transport components, usually exist in hypoxic tissue (Van Toai and Bolles, 1991).

Superoxide Dismutase is a free radical scavenging enzyme, which has also been included in the catalogue of anaerobic polypeptides (Monk *et al.*, 1987). The activity of SOD consistently increased in hypoxic as well as in 48 h post-hypoxic sample, with a maximum of 3-fold enrichment in their activity from control plants in Desla brown (Pandey *et al.*, 2000).

One protective system to anoxia tolerance involves SOD converting superoxide radicals to hydrogen peroxide, which is reduced to water by peroxidase or catalases. In anoxia tolerant rhizomes of *Iris pseudacorus*, SOD activity increased

some 13-fold during 28 days of anoxia but failed to increase in *Iris germanica* and *Glyceria maxima* which tolerate anoxia for much shorter periods (Armstrong *et al.*, 1994). This suggests that under anoxia; enzymes are induced that have a role in protection of cell metabolism from a subsequent exposure to free radicals.

Peroxidase activity was lower in flood tolerant variety FR 13A than in other varieties of Rice (Devender Reddy and Mittra, 1985). Bin Yan (1996) suggested that O_2^- induced lipid peroxidation and membrane damage, and that excessive accumulation of O_2^- is due to the reduced activity of SOD under flood stress. Zhou *et al.* (1997) reported at waterlogging at the seedling and stem elongation stages of winter rap caused a significant decrease in SOD and catalase activity.

Catalase (CAT) which is localised in the peroxisomes of higher plants, functions in the decomposition of H_2O_2 , which is also produced outside the chloroplasts by H_2O_2 - generating oxidases in the peroxisomes (Tolbert, 1971). Despite restricted localization, it may play a significant role in removing H_2O_2 can readily diffuse across the membranes and enter the chloroplasts (Bowler *et al.*, 1992).

After waterlogging for 6 days, the activity of SOD of wanmai wheat decreased noticeably, while it took longer to decrease in Yangmai wheat (Youngping *et al.*, 2000). There is some evidence that in flooding tolerant species, increased SOD activity under anoxia is an important protection mechanism to reverting oxidative damage during recovery from anoxia stress, e.g., after transient flooding (Crawford, 1993).

2.11. Molecular mechanism

2.11.1. SDS-PAGE-Anaerobic root proteins

Anaerobic stress induces a rapid change in gene expression in plants. In maize, for example, a set of 20 polypeptides designated as ANPs (anaerobic proteins); is selectively synthesized in the primary root (Nover, 1989). Anoxia induces a similar

pattern of altered gene expression in barley (Hoffman *et al.*, 1986) and Totton Wood (Kimmerer, 1987).

Sudden changes in root oxygen partial pressure immediately lower the energy status of cells and thus give little opportunity for induction of an alternative metabolism. Despite this, in anoxic roots of maize, there is a remarkable change in the pattern of protein synthesis detected on PAGE plates (Sachs *et al.*, 1980). Some 20 anaerobic polypeptides can be distinguished in maize, including alcohol dehydrogenase (Ferl *et al.*, 1980), pyruvate decarboxylase (Lazlo and Lowerence, 1983), glucose phosphate isomerase and adolase (Kelley and Freeling, 1984a) and glyceraldehyde-3-phosphate dehydrogenase (Russel and Sachs, 1992). All of these enzymes are essential to glycolysis and fermentation. Among the many ANPs, alcohol dehydrogenase (ADH) and pyruvate decarboxylase are often most abundant.

Mujer *et al.* (1993) reported that in the intolerant species, *Echinochloa colonum* and *Echinochloa crus-paronis* to polypeptide were enhanced or repressed under anoxia whereas in the intolerant *Echinochloa* species and rice, a total of atleast 9 to 13 anaerobic stress proteins and 4 to 7 aerobic proteins were noted.

The reduction in the number of an aerobically inducible soybean genes relative to the maintain consistent with *in vivo* and *in vitro* protein synthesis (Russel *et al.*, 1990). The pattern of protein synthesis has been compared in the different organs of maize (*zea mays*) using 2-D gel electrophoresis (Okimoto *et al.*, 1980).

In maize roots, a more complex pattern of proteins are synthesized under hypoxia than anoxia, some proteins are glycolytic enzyme, whereas others are hypoxia-specific responses (Bennett and Freeling, 1987). The coordinate expression of the ANP is accomplished by a common transacting factor that interacts with an anaerobic responsive element (ARE) in the promoter region of each gene (Olive *et al.*, 1991).

2.11.2. Isozymes

Multiple forms of peroxidase (POD) existed in many plant tissues (Vanhuystee, 1990). Anionic isoforms were localized in cell walls while basic peroxidases in the vacuole (Schloss *et al.*, 1987).

Several POD isoforms were found in the extracellular matrix. Ascorbate peroxidase (APX) were the most important enzymes for protection against H_2O_2 in the cytosol and chloroplasts (Asada, 1992) particularly in the "Mehlar reaction". The O_2^- produced in the "Mehlar reaction" will be dismuted to O_2 and H_2O_2 by SOD. Thus, effective scavenging of active oxygen species require concerned action by SOD and APX.

Under water stress there was a potential increase in the POD activities (Zhang and Kirkam, 1999), while it decreases in maize (Wang *et al.*, 1995). In the cytosol of nitrogen fixing root nodules, where Reactive Oxygen Species (ROS) are produced under stressed conditions also it may constitute upto 1% of the total protein. Ascorbate peroxidases use ascorbate as a substrate and are believed to scavenge excess of H_2O_2 formed in plant cells under both normal and stress conditions. The product of ascorbate oxidation by ascorbate peroxidase is an ascorbate free radical which is reduced back to dehydroascorbate by the enzyme monohydroascorbate reductase with NADP as the electron donor (Srivastava, 1999).

2.12. Anatomical studies

2.12.1. Hormone Metabolism - Aerenchyma development

Adaptive mechanisms to secure a renewed supply of oxygen to flooded root tissues included the development of internal gas filled channels called aerenchyma, that allow oxygen movement form the aerobic shoot to the anaerobic root (Jackson, 1989).

Aerenchyma differs in origin among species and may be either lysigenous or schizogenous. Lysigenous aerenchyma develop as a consequence of senescence of specific cells followed by their autolysis and disintegration, whereas schizogenous aerenchyma develop by cell separation and cell division (Kawase, 1981).

The accumulation of ethylene in soil as well as in roots under waterlogging is well documented. Accumulation of ethylene in soils becomes increasingly important of oxygen concentrations in the soil atmosphere below 9% (Hunt *et al.*, 1981). Because of the much lower rates of diffusion of gases in water as compared with air, the water film around roots entraps ethylene in the root tissue (Water Jacket effect).

Many of the adaptive growth response in hypoxic roots and shoots occur in response to ethylene. Ethylene accumulates in flooded soils and in submerged plant parts to concentrations of 10 ml L^{-1} (Musgrave *et al.*, 1972). First, the diffusion of ethylene from the root into the water is 10^4 times slower than its diffusion into air (Stunzi and Kende, 1989). This ethylene may be released into the internal aerenchyma channels and diffuse from the root to the shoot. Secondly, the synthesis of ethylene in the hypoxic root and in the aerobic shoot is increased. Various studies subsequently confirmed that the ethylene levels in flooded plants generally exceed those found in drained plants (Jackson and Campbell, 1975a).

In a wide range of upland plants, the prominent responses are the formation of lysigenous aerenchyma as observed in *zea mays* (Kuo, 1993), fast under water elongation of roots or leaves of aquatic or amphibious species, and stomatal closure and epinastic leaf curvature, each of these reaction is mediated by plant hormones with ethylene and abscisic acid playing prominent roles (Jackson *et al.*, 1993; Brailsford *et al.*, 1993).

Ethylene is the principal mediator, promoting the development of aerenchyma in maize as well as other plants (Jackson, 1990a and 1990b). Hormonal regulation is

based on an increase of the concentration of ethylene, which interacts with gibberellin (Kende, 1987) and auxins (Horton, 1987). Auxins and gibberellins are prerequisites for ethylene action and play triggering rather than regulatory functions. Gas diffusion into the plant is impeded, leading to low oxygen partial pressure. The low partial pressure stimulates biosynthesis of ethylene by increasing ACC (1-aminocyclopropane-1-carboxylic acid) synthetase activity, thus further enriching the stem with ethylene (Pearce *et al.*, 1992).

Formation of gas spaces may be mediated by enhanced ethylene concentration under anaerobiosis (Konings, 1982). A higher ethylene concentration under flooding increases the sensitivity of adventitious root-forming tissues to endogenous auxins (Visser *et al.*, 1996). Bacanamwo and Purcell (1999) evidence that flooding acclimation in soybean relying on N₂ fixation is dependent on adventitious root and aerenchyma formation.

Changes in the root anatomy in response to flooding are closely correlated with changes in root morphology. After waterlogging many old roots die, but numerous adventitious roots with well-developed aerenchyma emerge from the base of the stem and grow to a limited extent into the anaerobic soil. Whether existing roots die to sudden waterlogging (anaerobiosis) and new (adapted) roots have to be formed, or whether the development of aerenchyma is enhanced in the existing roots, depends mainly on the plant species and the flooding tolerance of the species (Laan *et al.*, 1991).

In *Rumex* species, changes in the distribution of the rooting system in the soil in response to flooding have been correlated with the relative flooding tolerance of different species. The more flood tolerant *R. crispus* and *R. palustris* developed new flood-resistant roots in the upper 10 cm of waterlogged soil, whereas the flood susceptible *R. acetosa* did not change its vertical root distribution (Roesenek *et al.*,

1992) Roots of flooded plants tend to become negatively gravitropic, or in other words, they grow upwards (Wample and Reid, 1978).

Adventitious roots are usually desired from localized excessive phellogen activity that produces strongly hydrophobic lenticels on the surface of stem. If the lenticels are below the water level, roots will emerge. However, if the lenticels are above the waterline, they will enhance gas diffusion into stem but will not produce roots (Nilsen and Orcutt, 1996).

In cotton plant, as a result of waterlogging the root apical meristem the pericycle remains capable of giving rise to new lateral roots, when oxygen concentration rises again (Ray and Khadder, 1983). Topa and McLeod (1986) suggest that the flooding tolerance of pine seedlings is positively correlated with a greater ability of these pines to produce new secondary or adventitious roots.

The effects of hormones (ethylene in relation to the morphological and anatomical modifications) in response to soil waterlogging or anoxia and submergence have been discussed extensively (Jackson and Drew, 1984; Jackson and Pearse, 1991).

Secondary aerenchyma formation in hypocotyls of summer leguminous crops grown under upland and excessive moisture conditions (Mochizuki *et al.*, 2000). Under well saturated soil conditions formation of lysigenous intercellular spaces in the root cortex were observed in *Glycine max*, *Vigna angularis*, *Vigna mungo*, *Vigna radiata*, *Vigna umbrellata* (Shimamura *et al.*, 1996).

Visser *et al.*, (2000) concluded that aerenchyma is not constitutive in the *Carex* species under study and is best developed in *Carex* species from wetlands species with less aerenchyma perform poorly when soil flooded, but conditions of partial

submergence could even affect species with a considerable amount of root aerenchyma.

2.13. Yield and yield parameters

The productivity of many agricultural crops are adversely affected when the soil is either temporarily or continuously flooded (Kozlowski, 1984).

Oosterhuis *et al.* (1990) reported that final seed yields were reduced significantly by a mean of 52 and 40% for Essay and Forrest cultivars of soybean. Inadequate oxygen supply for root respiration is the main cause of reduced yield under waterlogged conditions (Russel, 1977). Waterlogging has been reported to affect seed per pod and seed per plant (Griffin and Saxton, 1988). Results demonstrate that pods per reproductive node and indicators of branch development (Board *et al.*, 1990) can be used as criteria to quantify genetic and environmental responses to waterlogging. Waterlogging for 7 days caused greatest yield loss for late-planted soybean when stress was applied at early reproductive stages (Linkemer *et al.*, 1998).

Yield decline was due to the reduction of different yield components, depending upon the developmental stage and time when the waterlogging occurred (Boem *et al.*, 1996). Average yield of blackgram declined from 12 per cent to 36.5 per cent as the duration of excess water was prolonged from 24 h to 120 h (Singh *et al.*, 1987). Pod yield per plant of soybean was reduced by 7 to 10 and 12 to 33 per cent with waterlogging of 4 days and 8 days respectively (Sorte *et al.*, 1996).

Waterlogging at the seedling, vegetative and preflowering stages decreased the number of nodes and thus also the number of pods per plant of peas waterlogging at the pod-filling stage decreased the proportion of flowering nodes that bear fruit (Cannell *et al.*, 1979).

Yadav and Saxena (1998) reported that the deleterious effects of waterlogging on various growth attributes and seed yield were partially alleviated by higher dry matter accumulation in tolerant genotypes of mungbean.

Belford *et al.* (1980) found that flowers and pods per plant was the major cause of yield loss from flooding of pea. Increasing the flood period from 24 to 72 h resulted in a significantly lower yield response in soybean five of the six cultivar-year situations while increasing the flooding period for 24 to 48 did not affect yield response in any situation (Heatherly and Pringle, 1991). The difference in soybean grain yield due to waterlogging of 24 and 28 h was significant which increased substantially as duration of excess water prolonged to 72 or 120 h (Singh and Singh, 1993).

Three years pooled data indicated that persistent waterlogging of 4 days and 8 days significantly reduced the grain yield of soybean by 8 to 11% and 13 to 13% respectively over control during vegetative, flowering and pod development stages (Sorte *et al.*, 1997).

Cowie *et al.* (1996) reported that waterlogging at 21 DAS of chickpea reduced branch number, shoot yield and pod number by 30-35% in comparison with controls, while seed number per pod, weight per seed and harvest index were unaffected. Krishnamoorthy *et al.* (1983) recorded a 56% reduction in the number of seeds produced after chickpeas were waterlogged for 12 days from three weeks after sowing.

Seed yield of chickpea cv. H-75-35 was affected more by waterlogging at flowering than during vegetative growth and pod filling (Bisoni and Krishnamoorthy, 1991). Jhorar *et al.* (1995) observed that continuous flooding for 3, 6 and 9 days after 28 DAS caused about 48, 79 and 93 per cent decrease in yield of mungbean.

Sunflower yield was not affected by the anthesis waterlogging but no consistent effect on seed number or 1000 seed weight but in sorghum, yield was most reduced by the initiation waterlogging largely as a result of reduced seed number (Orchard and Jessop, 1984).

The reduction in rice yield was correlated with the decrease in number of panicles and the decrease in grain yield was more pronounced if plants were submerged for 12 days at early than that at late vegetative stage (Devender Reddy and Mitra, 1985).

Mochizuki and Matsumoto (1992) reported reduction in seed weight of soybean subjected to excessive flooding. Severe reduction in grain yield was caused by 8 days waterlogging mostly due to ill effect on various yield contributory parameters like pod weight, seed weight (Sorte *et al.*, 1995).

de Bruyn (1982) concluded that the highest seed cotton and lint yields were obtained from plants which did not undergo soil saturation at any time during the season as well as from plants that were subject to only moderate duration of saturation after the onset of flowering but were not saturated prior to flowering.

Excess water at flowering decreased yields of early maturing cultivars of soybean more than those of mid and mid-late maturing cultivars (Jin *et al.*, 1996). Differential response of wheat genotypes to waterlogging treatments has observed as vegetative dry biomass, straw and grain yields (Tarekegne *et al.*, 2000).

2.14. Chemical manipulation for induction of flooding tolerance

The Abscisic acid (ABA) or the ethylene precursor 1 aminocyclopropane-1-carboxylic acids (ACC) were as physiologically active messages from roots to the shoot of flooded plants and close stomata and promote petiole epinasty. These response are believed to minimize damage to shoot tissues of flooded plants by

reducing evapotranspiration at a time when root hydraulic conductance are small as a result of oxygen shortage (Everard and Drew, 1989).

Exogenous application of some growth regulators to waterlogged plants has given beneficial results. For instance, synthetic cytokinins applied to wheat seedlings at the onset of waterlogging delayed degradation of chlorophyll (Dong and Yu, 1984). The recovery process of sorghum seedlings after waterlogging was enhanced by foliar application of 5 ppm kinetin (Bhagwat *et al.*, 1986).

Konings and de Wolf (1984) tested the effects of applying different hormones to seedlings roots of maize. Pretreatment of groundnut cv M. 194 seedlings before waterlogging with B-nine (daminozide), CCC (Chloramequat) and phosphon-D (Chlorphosphonium chloride) at 5-50 mg/plant partially reduced the deleterious effects of waterlogging on number of nodules per plant, and the degradation of chlorophyll was fully dominated by B-Nine (Krishnamoorthy *et al.*, 1981). But in bengal gram application of CCC but not B-nine partially relieved the deleterious effects of waterlogging (Krishnamoorthy *et al.*, 1983). Gibberellin level falls after one or two days of flooding, and the exogenous application of gibberellic acid stimulates stem growth of the waterlogged plants (Reid and Crozier, 1971).

Jackson and Campbell (1971) reported that some of the waterlogging symptoms might be partly because of deficiency of endogenous Gibberellic acid (GA_3) and cytokinins. The inhibitory effect of 7 days of waterlogging was completely alleviated by 100 mg^{-1} of GA_3 given at vegetative stage of peanut. However, the beneficial effect of GA_3 , was partial when the duration of waterlogging was increased to 14 days (Bishnoi and Krishnamoorthy, 1992).

In maize, leaf diffusive resistance was not significantly affected with Benzyl adenine (BA) but spray of GA_3 reduced stomatal resistance resulting into more opening of stomata (Goswami and Jai Dayal, 1987). Drew *et al.*, (1979) studied that

spraying leaves with 6-Benzylaminopurine (BAP) resulted in small improvement in leaf extension and retarded the premature loss of chlorophyll in the older leaves after 7 days waterlogging.

Zhou *et al.*, (1997) reported that foliar sprays of mixatol at the flowering stage alleviated plant damage caused by waterlogging by retarding chlorophyll and nitrogen degradation increasing SOD and CAT activities and improving yield components and seed yield of waterlogged winter rape plants.

Bakhtenko and Platonor (1999) reported that a synthetic analogue of cytokinin altered the composition and ratio of phytohormones and increased the concentration of auxin as a result, the growth rates and yield of wheat increased. Wang *et al.* (1997) observed that foliar application of 10 ppm BAP improved resistance to waterlogging by decreasing leakage of cell electrolytes, chlorophyll catabolism and increasing SOD activity.

Leslie and Romani (1988) indicated that endogenous salicylic acid plays a whole in ethylene regulation in plants or that some effects of exogenous Salicylic acid (SA) application were mediated by altered ethylene. Metabolism remains to be explored.

Exogenous application of salicylic acid to plant has been shown to influence a wide variety of biological processes including stomatal function, flower stimulation, vegetative bud formation, adventitious root initiation, disease resistance and heat production (Raskin, 1992). Increased yield of mungbean (Singh and Kaur, 1980) due to salicylic acid treatment was reported.

Brassinosteroids (BRs) were found to increase the yield of wheat under stress conditions (Sairam, 1994). Bhatia and Kaur, (1997) recorded highest yield from homobrassinolide treated mungbean plants (higher pod number per plant). Fujii *et al.*

(1991) found that brassinosteroids increased translocation resulting in increased grain number per panicle and harvest index which contributed to higher grain yield of rice. Ramaraj *et al.* (1997) reported that homobrassinolide was most effective for improving yield of various crops including rice.

Brassinosteroids are a novel group of plant hormones that regulate cell division, cell expansion, cell division, reproductive and vascular development, retard leaf abscission and enhance resistance to stress (Adam, 1994).

Exogenous application of BRs inhibits primary root extension and lateral root formation, with occasional promotion of elongation or adventitious rooting seen with ppm concentrations (Sasse, 1994). Gomathi, (1996) noticed that brassinosteroid application in soybean increased the seed and pod number through an increase in fertile node number and number of pods per fertile node. Brassinolide application increased 100 seed weight in groundnut (Vardhini and Rao, 1998).

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out primarily to screen the genotypes of pulses for flooding tolerance and to understand the physiological, biochemical and molecular mechanisms of flooding tolerance. An attempt was also made to induce the tolerance with chemicals and plant growth regulators. This research work was carried out in a series of experiments comprising laboratory, glass house and field trails. A brief account of the materials used and methodologies followed in different experiments in the present study are given below.

3.1. Experimental Materials

Pulse crop as listed in table 1 are collected from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were used for the study.

3.2. Experiment: I Lab studies – Screening pulses for flooding tolerance

3.2.1. Screening methods

Techniques developed by Nelson *et al.* (1983) were followed for the study. Twenty-five seeds of each pulse genotype were germinated and grown inside a rolled moist paper towel. After 7 days, 3 seedlings from each pulse genotype were selected for homogeneity of primary root length. The screening methods were, (i) Triphenyl Tetrazolium Chloride (TTC) reduction method, (ii) Electrical Conductivity (EC) test – relative injury test, (iii) root Alcohol Dehydrogenase Activity (ADH) and (iv) Pressure Chamber (PC) method (water potential of leaves)

Table 1. Details of pulses selected for study

Crop	Variety	Duration (days)	Parentage
Greengram	CO 4	85	Mutant of CO1
	CO 5	70-75	Hybrid derivative of KM2 x MG
	CO 6	62-67	WGG 37 x 105
	KM 2	65-70	Hybrid derivative No. 127 x S.9
	VBN 1	65	Hybrid derivative of S.8 x PIMS 3
Black Gram	CO 5	70-75	Pure selection from Musiri
	VBN 3	65-70	LBG 402 x LBG 17
	ADT 5	62	Pureline selection from Kanpur variety
Red gram	CO 5	110	Mutant CO 1
	CO 6	170-180	Mutant of SAI
	VBN 1	95-100	(Prabath x NY3C) x (T21 x ICP 302)
	COPH 2	120-130	MS CO 5 x ICPL 83027
Soybean	CO 1	85	Reselection from a Thailand variety
	CO 2	85-90	UGM 21 x JS 335
Bengal gram	CO 3	85	Pureline selection from Maharashtra collection
	CO 4	80-85	ICCC 42 x ICC 12237
Cowpea	CO 2	90	Hybrid derivative (CS 21 x C 49)
	CO 4	85	Selection form Russian Giant
	CO 6	65-70	MS 9804 x C 152

3.2.1.1. Triphenyl Tetrazolium Chloride (TTC) reduction method

The root segments from one of the 3 seedlings were selected as control (non-stressed) samples and were immediately treated with TTC. The remaining 3 segments from each of 2 seedlings were stressed to simulate flooded conditions by submersion in 10 ml of distilled water and placed in a dark room at room temperature (22°C). The method for TTC treatment was similar to that of Palta *et al.* (1981) briefly, the root segments were transferred to a desired time to test tubes containing 3 ml of 0.08 per cent TTC solution in 0.05 M phosphate buffer, (pH 7.4). The samples were incubated for 24 hours in the dark at 22°C and then reduced TTC was extracted. The reduction of TTC was estimated at 485 nm with a spectrophotometer with reference extract from the nonstressed controls. Injury was expressed as the per cent decrease in TTC reduction as compared to the control.

3.2.1.2. Electrical Conductivity (EC) test – Relative Injury test

The root-segment samples were prepared as for the TTC test. Immediately after excision, all segments were individually transferred into 30-ml vials, submerged in 10 ml of distilled water and the electrical conductivity of the effusate in each vial recorded after the samples had been gently shaken for 1 h. This reading was taken as the non-stressed reference level. All vials thereafter remained undisturbed in a dark room at room temperature (22°C). After 8, 12, 24 and 36h intervals following the initiation of flooding, conductance was read to determine the amount of ion leakage. After final measurement, following 36 h of root submersion, the vials containing 10 ml of solution and root segment, were quickly frozen to -20°C and were then thawed by transfer to tap water at 25°C to kill the tissue and releases all the ions into solution. The conductivity reading of the killed tissue gave the total ion content of tissue. The amount of ion leakage during flooding was determined by dividing the 8, 12, 24 and 36h reading (R_1) by reading of the killer tissue (R_2). The ratio of the two readings (R_1/R_2) was used as a measure of the relative injury.

3.2.1.3. Alcohol Dehydrogenase activity (ADH) (Russel *et al.* 1990)

The root samples were prepared as for the TTC and EC Test. Fibrous roots were homogenized in a ice cold mortar by using 2 ml g⁻¹ of cold extraction buffer (50 mM Tris-Cl (pH 6.8), 15% V/V glycerol, 5% V/V β-Mercaptoethanol, and 1 mM PMSF). The homogenate was centrifuged at 10,000 rpm for 15 min at 5°C. The supernatant was collected and used for assay.

The enzyme reaction mixture contained 0.300 ml of 1 M Tris-Cl (pH 8.0), 0.040 mL of 10 mg/ML NAD⁺, 10 to 20 μL of sample proteins extract, and water to 2 ml volume. The mixture was inverted three times, placed in the spectrophotometer and adjusted to 0.000 at A₃₄₀. Adding 0.040 ml of 50% ethanol started the assay. After mixing, the A₃₄₀ was recorded every 15 seconds from 15 to 75 sec. and every minute thereafter for 3 min. Data were checked for linearity overtime. The data were converted to ADH activity units. The unit was defined as the ΔA₃₄₀ of 0.001 per min. ie. μmoles NADH min⁻¹ mg Pro⁻¹.

3.2.1.4. Pressure Chamber (PC) method

From each of the pulse genotypes, uniform seeds were planted 2 each in 10 cm diameter pots. After 15 days, the flooding treatment was produced by inserting the pots containing the plants into 16 cm diameter sealed plastic pots. Tap water was added to the outer container and maintained at a level of 9 cm below the surface of the soil throughout the experiment. After 5 days, each pulses plant was removed from the chamber and a pressure chamber was used to measure the xylem water potential of the 3 largest leaves. Leaf water potential was measured by using pressure chamber (Soil moisture Equipment corp., P.O. Box 20025, Santa Barabara, (A 93105, U.S.A.) between 11.00 A.M. to 1.00 P.M. every alternate day during the stress period and expressed as MPa. For this purpose, leaf sampling and preparation consisted of excising the most recently fully expanded leaf placing it in a small plastic bag to reduce loss of water during measurement. The leaf is kept inside the pressure vessel in such a way that the cut end of leaf is seen above the chamber. Using a knob gradually

increases the pressure inside the chamber and the xylem sap is oozed out. Usually compression is done using dry hydrogen gas from a cylinder. Oozing of Xylem sap is detected with a hand lens. The pressure that results in oozing of xylem sap is recorded as water potential.

3.3. Experiment II. Pot culture studies

Studies on physiological, biochemical and molecular mechanism of flooding tolerance were carried out through glass house experiment. Based on the results of laboratory screening methods, the tolerant and susceptible pulses genotypes used in the pot culture experiment at the glass house of Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The experiment details are given below,

Crops used	-	a. Cowpea (CO 2 and CO 4)
		b. Soybean (CO 1 and CO 2)
		c. Green gram (CO 4 and VBN 1)
Design	-	FCRD
Replication	-	Three
Treatments	-	Seven

3.3.1. Treatment details

It consists of three pulses and each pulses have 2 genotypes (Tolerant and susceptible). The treatment imposed to each pulse genotypes. The treatment details are given below,

1. Flooding at vegetative stage for 5 days
2. Flooding at vegetative stage for 10 days
3. Flooding at flowering stage for 5 days
4. Flooding at flowering stage for 10 days
5. Flooding at pod filling stage for 5 days
6. Flooding at pod filling stage for 10 days
7. Control (no flooding)

3.3.2. Experimental setup or preparation of pots

Pot mixture was prepared by mixing thoroughly two parts of soil and one part each of well-decomposed farmyard manure and sand and filled in pots of size 28 x 30 cm with 10 kg of soil in each pot. For flood treated pots, the innerside of the pot is coated with wax for preventing water leakage. Five seeds of screened pulse genotypes were sown in 5 spots per pot and later as thinned to have a uniform population of three plants per plot. A basal dose of N and P were applied at the rate of 25 and 50 kg ha⁻¹ in the form of urea and superphosphate respectively. Normal plant protection measures were adopted through out the crop growth period.

3.3.3. Soil characteristics

The physical and chemical properties of the soil used for pot culture and field experiment are given in Table 2.

Table 2. Soil properties of the experimental field

Particulars	Pot culture experiment	Field experiment
Physical properties		
Field capacity (%)	14.80	16.50
Bulkdensity (cm ³)	1.42	1.58
Permanent wilting point	7.50	8.70
PH	7.1	6.8
EC (m. mhos. cm ⁻¹)	0.2	0.3
Chemical composition (kg ha⁻¹)		
Available nitrogen	241	283
Available phosphorus	16	18
Available potassium	421	573

3.3.4. Flooding stress imposition

Soil moisture content was recorded gravimetrically. Flooding stress or treatments were imposed at vegetative, flowering and pod filling stages. The pots were filled with water, the flooding height was maintained at 3 cm above the soil level for 5 and 10 days at 100 per cent field capacity (ie fully saturation of soil) respectively, after which the stagnant water in the pots were drained. Control pots received normal irrigation throughout its life cycle (15 to 16 per cent field capacity).

3.3.5. Sampling for observation

Plant samples were drawn at vegetative, flowering and pod filling stages to the end of the flooding treatment for recording various morphological, physiological, biochemical and anatomical characters. Yield parameters were recorded for different growth stages at harvest.

3.3.5.1. Morphological characters

Plant height

The plant height was measured from the ground level to the tip of growing point and is expressed in centimeter.

Root length

Length of the root was measured from the base of the shoot to the tip of the longest root and expressed in cm.

Adventitious root numbers

Number of roots was arrived at by counting from the base to the tip of the taproot and expressed in numbers.

3.3.5.2. Growth Attributes

Leaf area

Leaf area was measured in a leaf area meter (LICOR, Model LI3000) and expressed in square cm per plant.

Specific leaf weight (SLW)

SLW was arrived at by using the formula suggested by Pearce *et al.* (1968) and expressed in mg cm^{-2} .

Specific leaf area (SLA)

Employing the formula of Kvet *et al.* (1971), SLA was calculated using leaf area and leaf dry weight and expressed in $\text{cm}^2 \text{g}^{-1}$.

Total Dry Matter Accumulation (TDMA)

The TDMA at each growth stages from each pot were recorded after oven dried at 85°C for 24 hours. They were expressed in g plant^{-1} .

3.3.5.3. Nodulation status

Nodule number

Plants were sampled for nodules at all stages and nodule number was recorded, the nodules were counted after washing of the adhering soil.

Nodule Dry weight

The nodule dry weight was arrived at by weighing dry nodules and was expressed in mg.

3.3.5.4. Physiological Changes

3.3.5.4.1. Leaf Gas exchange measurements

Gas exchange parameters viz., Photosynthetic rate ($\mu \text{ mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\mu\text{g mole H}_2\text{O m}^{-1} \text{ s}^{-1}$) and stomatal conductance (cm s^{-1}) were recorded using an advanced portable CO_2 gas analyzer (model CL - CID Inc, USA). The readings were taken during 11:00 to 12:00 noon on clear sunny day when the PAR was more than $1000 \mu \text{ moles m}^{-2} \text{ s}^{-2}$. Third fully opened single trifoliolate leaves from the top were damped to the leaf chamber and held perpendicular to the incident light and only the computed values were taken. The instrument was working in open type principle thus maintaining a constant CO_2 flux to the leaf chamber, which is at

52
ambient concentration. RH was also maintained at a steady state level equal to the ambient RH to simulate a condition very similar to that of the ambient air.

3.3.5.4.2. Leaf water potential

The leaf water potential was measured as per the procedures described in the experiment I.

3.3.5.4.3. Chlorophyll Fluorescence

Plant Efficiency Analyzer (Hansatech, UK) (PEA) (Krause and Weis, 1984).

Chlorophyll Fluorescence measured by the system called Plant Efficiency Analyzer (Hansatech, UK) (PEA).

The key fluorescence parameters of F_o , F_m , F_v and the ratio of F_v/F_m are automatically calculated using light level used during measurement.

$$\frac{F_v}{F_m} = \frac{\text{Ratio of variable fluorescence}}{\text{Maximum fluorescence}}$$

F_v/F_m is a useful ratio that has been proportional of quantum yield and show a high degree of Photosynthesis.

F_m is the time at which maximum fluorescence occurs.

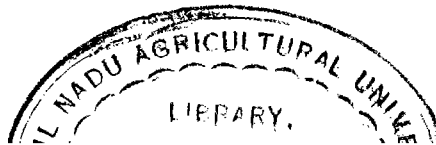
3.3.5.5. Biochemical Mechanism of Flooding Tolerance

3.3.5.5.1. Leaf soluble protein and Root anaerobic proteins

The soluble protein was estimated by measuring the colour developed by the reduction of Folin-Ciocalteu reagent, following the method of Lowry *et al.* (1951) and expressed in mg g^{-1} fresh weight.

3.3.5.5.2. Chlorophyll content

The chlorophyll content was estimated in fully expanded trifoliolate leaf (3rd leaf from top) in the main branch at the specified stages (Yoshida *et al.*, 1976), and expressed in mg g^{-1} fresh weight.



3.3.5.5.3. Nitrate - reductase activity

The third leaf from the top was used for estimating the nitrate reductase (NR) activity in all three stages. NR activity was estimated by using the method described by Nicholas *et al.* (1976) and the enzyme activity was expressed in μ moles NO_2 produced $\text{g}^{-1} \text{h}^{-1}$ fresh weight.

3.3.5.5.4. Carbohydrate fractions

- i. Total carbohydrate was estimated using anthrone reagent as per the procedure Hedge and Hofreiter (1962) after hydrolyzing the leaf samples with HCl and expressed as mg g^{-1} fresh weight.
- ii. Starch content was estimated by extracting samples in perchloric acid - 52 per cent (Hedge and Hofrieter, 1962) after removing sugars by repeated washing high with methanol (80 per cent) and the content was expressed as mg g^{-1} fresh weight.

3.3.5.5.5. Nitrogenase activity

The nitrogenase activity of the root nodules was determined by acetylene reduction method as described by Hardy *et al.* (1968). Nodulated root system was gently freed from soil particles, washed briefly in running tap water and dried by blotting with filter paper. The nodules were then transferred to serum bottles of 15 to 20 ml capacity. The container was closed air tight with rubber stopper through which gas injection and gas sampling were done using microsyringe. The air in the container was evacuated and then an equal quantity of acetylene was injected into it and the reaction was allowed to proceed for 1 h at $28^\circ\text{C} \pm 2^\circ\text{C}$, after which 0.5 ml gas sample was with drawn and fed to the gas chromatograph (Perkin Elmer - F33 model) having poropak column 80-100 mesh, column temperature 150°C and oven temperature 95°C .

The acetylene reduction activity was graphically represented on the gas chromatography chart. It was quantified by measuring the height of the peak in mm.

The results were compared with that of a standard graph and the nitrogenase activity in terms of ethylene produced was computed as follows.

$$\text{Nitrogenase activity} = \frac{\text{Peak height} \times \text{Range} \times \text{Attenuation} \times \text{volume of the gas in the tube} \times 0.01}{\text{Hrs of incubation} \times \text{Dry weight of nodule (g)} \times \text{sample injected (ml)}}$$

3.3.5.5.6. Leghaemoglobin content of root nodules

The leghaemoglobin content of control and flooded root nodules was determined by following the method advocated by Schiffonan and Lobel, (1970). The washed and weighed nodules were crushed in Tris-acetic acid buffer and centrifuged at 3000 rpm for 10 minutes. The supernatant was made up to 4.0 ml with tris buffer and 2.0 ml of benzidine reagent was added. The optical density was measured at 540 nm in spectronic 20 using Draphkins reagent as blank. The quantity of leghaemoglobin was calculated by referring to standard graph and was expressed in mg per gram of dry nodule.

3.3.5.6. Ethanol Fermentation Enzymes

3.3.5.6.1. Alcohol Dehydrogenase (ADH) activity

The activity of ADH were estimated as per the procedures described in the experiment I.

3.3.5.6.2. Pyruvate Decarboxylase (PDC) activity

Principle

Pyruvate decarboxylase is assayed by a method in which decarboxylase is coupled with alcohol dehydrogenase in the presence of thiamine pyrophosphate and NADH, The rate of disappearance of NADH is followed spectrophotometrically at 340 nm (Oba and Uritani, 1982).

Reagents

Tris - HCl buffer, 0.25M, pH 6.5 at 25°C

Thiamine Pyrophosphate (Tpp) 14 mM, stored at - 20°C

Yeast alcohol dehydrogenase, 5mg/ml, stored at - 20°C

NADH, 6.7 mM, prepared freshly

Procedure

In a reference quartz cell, are placed 0.08 ml of buffer, 0.02 ml of Na pyruvate, 0.005 ml of enzyme extract and water to make the final volume of 0.4 ml. In a test quartz cell, are placed 0.08 ml of buffer, 0.01 ml of TPP, 0.003 ml of alcohol dehydrogenase, 0.014 ml of $MgCl_2$, 0.010 ml of NADH, 0.005 ml of enzyme extract, and water to make the final volume of 0.38 ml. When the decrease in absorbance at 340 nm ceases, the reaction is initiated by the addition of 0.02 ml Na pyruvate. The rate of decrease in absorbance at 340 nm is followed in a recording spectrophotometer.

One unit of enzyme activity is defined as the amount of enzyme required to produce 1 nmol of acetaldehyde per minute under the above assay condition based on the fact that 1nmol of acetaldehyde yields 1nmol of NAD^+ by defined as the number of enzyme units per milligram of protein.

3.3.5.7. Malate dehydrogenase (MDH) activity (Sadasivam and Manickam, 1996)

Malate dehydrogenase (MDH) is one of the enzymes catalyses the reversible conversion of oxaloacetic acid to malic acid. Since it is an oxidoreductase involving nicotinamide adenine dinucleotide the decrease in absorbance due to the oxidation of NADH is followed.

Enzyme extract

The root tissue was thoroughly macerated with acid washed sand in a pre-chilled pestle and mortar in grinding medium (1ml/1g tissue) containing 50mM Tris-HCl (pH 8.0); 50mM $MgCl_2$, 5mM 2-Mercaptoethanol and 1mM EDTA. The

homogenate has passed through 4 layers of cheese cloth. The filtrate was centrifuged at 3000g for 20 min at 5°C. The supernatant was used as enzyme source.

Materials

Oxaloacetic acid (5 μ mole) 66mg/50 ml distilled water

Magnesium chloride (10 μ mole) 203.5 mg/50ml distilled water

Tris-HCl Buffer (0.1 M) pH 7.8

NADH (0.4 μ moles) 5.32mg/20ml

Procedure

All the reagent were pipetted as shown below

S.No.	Reagents	Test (ml)	Blank (ml)
1.	Oxaloacetic acid	0.5	0.5
2.	MgCl ₂	0.5	0.5
3.	Tris-HCl (pH 7.8)	1.3	1.8
4.	Enzyme extract	0.2	0.2
5.	NADH	0.5	-

The spectrophotometer was set to zero absorbance at 340 nm without adding NADH in the test against blank in the reference cuvette. NADH was added quickly as possible into test, mixed and recorded the initial absorbance. The absorbance was recorded every 30 sec. for at least 3 min.

The enzyme activity was calculated as follows with decrease in absorbance for one min.

μ moles NADH oxidised per 0.2 ml enzyme extract

= Absorbance decrease per min \times 0.1613 \times 3 (Volume of the reaction mixture in ml)

3.3.5.8. Free radical scavenging Enzymes

3.3.5.8.1. Catalase

Catalase activity was determined following the method of Luck (1974). One gram of the sample was extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of the extract was added to the experimental cuvette containing 3 ml H₂O₂ - PO₄ buffer. The time taken for per cent change in absorbance (Δt), at 240 nm was recorded for calculating the enzyme activity and expressed as enzyme units g⁻¹ min⁻¹ tissue. All the operations were carried out at 0-5°C.

3.3.5.8.2. Peroxidase

Peroxidase activity was determined by adopting the method of Malik and Singh (1980). One gram of sample was extracted in 0.1 M phosphate buffer (pH 7.0). A known volume of the extract was added to an experimental cuvette containing 3 ml phosphate buffer and 0.05 ml guaiacol reagent and then 0.03 ml of H₂O₂ solution was added rapidly and the increase in absorbance at 436 nm was recorded. This Δt in minutes was used to calculate the activity. The enzyme activity was expressed as enzyme units per litre. All the operations were carried out at 0-5°C.

3.3.5.8.3. Superoxide dismutase (SOD)

SOD activity was determined by using nitroblue tetrazolium (NBT) salt as described by Beau Champ and Fridovich (1971) and expressed as enzyme units mg⁻¹ protein min⁻¹. The reagents used in the enzyme assay are.

Reagents

	Stock	Working solution	Total volume
1.	Riboflavin 200 μ M (100x) 3.8 mg/50ml	2 μ M	30 μ l
2.	Buffer 250 mM (5x) KH ₂ PO ₄ 3.402g/100ml K ₂ HPO ₄ 4.354g/100ml	50mM	600 μ l
3.	Methionine 130 mM (10x) 0.9698 g/50ml	13MM	300 μ l

4.	EDTA 10mM (100x) 0.3722 mg/5ml	0.1mM	30 μ l
5.	NBT 7500 μ M (100x) (4.08 mg/5ml)	75 μ M	30 μ l
6.	Enzyme extract in Phosphate buffer (pH 7.8)	-	50 μ l
7.	Distilled water		
	i. for sample	-	1.96
	ii. Blank (NBT- enzyme extract)	2.04	-
	iii. Reference blank (Enzyme)	-	2.01

The absorbance was measured at 560nm

Calculations

Absorbance of reference blank = x

Absorbance of sample = y

$$\frac{x - y}{x} \times 100 = Z \% \text{ inhibition}$$

$$50 \text{ per cent inhibition} = \frac{z}{50} = A \text{ unit}$$

i.e. 50 μ l enzyme extract yielded A unit

1000 μ l yields $A/50 \times 1000 = B$ unit

$$\frac{B}{\text{protein value}} = \text{SOD assay value (Enzyme units mg}^{-1} \text{ protein)}$$

3.3.5.9. Molecular studies

3.3.5.9.1. Electrophoresis (SDS - PAGE) (Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis)

SDS-PAGE analysis was done to identify the Anaerobic polypeptides under flooding condition as per the method of Laemmli (1970) with slight modifications as per the need given by Sadasivan and Manickam (1996).

A known quantity of leaf and root sample was homogenized in phosphate buffer (pH 7.0) in a pre chilled pestle and mortar and the homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was used for analysis.

Materials

Stock acrylamide solution

Acrylamide	30g
Bisacrylamide	0.8g
Water	100 ml

Separating gel buffer (pH 8.8)

1.875 M Tris - HCl	22.7
Water	100 ml

Stacking gel buffer (pH 6.8)

0.6 M Tris - HCl	7.26 g
Water to	100 ml

Polymerising agents

APS	0.5g/20ml, freshly prepared
TEMED	Fresh from refrigerator

Electrod buffer (pH 8.2-8.4)

0.05 M Tris	12g
0.192 M glycine	28.8g
0.1% SDS	2g
Water to	2L

Sample loading buffer (5 X conc)

Tris HCl buffer (pH 6.8)	5ml
SDS	0.5g
Sucrose	5g
Mercaptoethanol	0.25 ml
Bromophenol blue (0.5% W/V)	1ml
Water to	10ml

SDS 10% solution (Stored at room temperature)

Protein stain solution

Coomassie brilliant blue R 250	0.1g
Methanol	40ml
Acetic acid	10ml
Water	50ml

Destainer

as above without the dye

Procedure

Thoroughly cleaned glass plates and spacers were assembled properly and were clamped in an upright position on a gel casting unit with 2 per cent agar on bottom to seal the chamber leak proof between glass plates. 10-15 per cent gels were tried to get the better resolution.

Separating gel	15% gel	10% gel
Stock acrylamide	20ml	13.3ml
Tris-HCl (pH 8.8)	8 ml	8 ml
Water	11.4ml	18.1ml
Degassed for 3-5 min and then added		
APS (5%)	0.2ml	0.2ml
10% SDS	0.4ml	0.4ml
TEMED	20 μ l	20 μ l

The gel solution was poured in the chamber between glass plates carefully leaving 4 cm from the top and a layer of distilled water was added on top of the gel. Allowed to polymerise for 30-60 min.

Stacking gel	4%
Stock acrylamide	1.35 ml
Tris-HCl (pH 6.8)	1ml
Water	7.5ml
APS (5%)	50 μ l
SDS (10%)	0.1ml
TEMED	10 μ l

After removing water from the gel, stacking gel (4%) was added, comb was placed and kept for polymerising (30-60 min)

After polymerization the comb was taken out and the gel plate was removed from the casting unit to the electrophoresis apparatus. Concentration of the protein sample were adjusted to 50-200 μg in a volume of 25-50 μl , by mixing with sample loading buffer, boiled for 2-3 minutes for ensuring complete interaction between proteins and SDS. The sample was cooled and carefully injected to each well with a microsyringe. Then the apparatus was filled with electrode buffer in both tanks, cathode was connected at the top and anode at the bottom and turned the DC power pack to a constant current of 15 mA initially till the dye front crossed the stacking gel. Then the current was increased to 30mA until the bromophenol blue reaches bottom of the gel (about 3 h). The gel was removed from the plates and immersed in staining solution overnight. After proper staining the gel was transferred to destainer with gentle shaking and changing the destainer frequently till appropriate visibility of the bands on gel. The gel was photographed.

3.3.5.9.2. Isozyme analysis

500 mg of leaf and root sample was homogenized in 1 ml phosphate buffer (pH 7.0) in a pre chilled pestle and mortar. The homogenate was centrifuged in a refrigerated centrifuge at 18,000 rpm for 15 min and the supernatant was used for analysis. Electrophoretic separation of isozyme was done with 7 per cent polyacrylamide gel without using SDS. Casting of the gel was done as described earlier, without using SDS anywhere. Loading of the samples was done in fridge and chilled electrode buffer without SDS was used. The power supply was set to a constant 15mA current and the running of the gel was done inside refrigerator.

Peroxidase (POX) (Sadasivam and Manickam, 1996)

Staining solution

Solution A

Benzidine	-	100 mg
Acetic acid	-	0.5 ml
Acetone	-	0.5 ml
Ammonium chloride	-	6% w/v

Solution B

H₂O₂ - 30% v/v

Solution C

Glacial acetic acid - 7% v/v

Procedure

The gel was incubated in the dark in solution A. After 30 minutes solution B was added drop by drop till blue coloured bands appeared. The gel was then fixed with solution C and photographed.

3.3.5.10. Ethylene content of roots

Roots from flooded and control plants were cut and transferred to 25 ml vials containing ambient air and sealed with serum caps. Accumulated ethylene including ethylene produced in response to the wound, was determined by withdrawing 2 ml gas samples using hypodermic syringe were drawn after 24 hours and injected to Gas Chromatography (GC) and compared with standard sample for retention time, height of peak and area covered by the peak and the ethylene concentration was computed using the formula below as, adopted by Sadasivam and Manickam (1996).

$$\text{Standard amount of ethylene (E) in } \mu\text{mol} = \frac{0.0446 \times Z \mu\text{l}}{\text{Peak height in mm} \times \text{attenuation}}$$

$$\text{Amount of ethylene evolved} = E \times \text{peak height of the sample in mm} \times \text{attenuation}$$

3.3.5.11. Morphological and anatomical studies

Formation of adventitious roots and cracking or swelling of stems was visually observed in each pulse varieties.

Root anatomy through microtome sectioning – Aerenchyma formation

Paraffin sectioning of the roots was done as described by Johansen (1940). For this purpose, roots were collected from the control and flooded pulses varieties those grown in the Glasshouse.

i) Fixation and washing

The roots were fixed in 5 parts of 30 per cent formalin, 5 parts of glacial acetic acid and 90 parts of 70 per cent ethanol for 29 h in 4°C. The roots were then transferred to 50 per cent, 60 per cent and 70 per cent ethanol each at one-hour interval.

ii) Dehydration

The roots were gradually dehydrated in tertiary butyl alcohol (TBA) series as shown in the Table.

Alcohol (%)	TBA (ml)	Ethanol (95%)	Distilled water (ml)	Duration (hrs)
50	1.0	4.0	5.0	2-3
70	2.0	5.0	3.0	12
85	3.0	5.0	1.5	12
95	4.5	5.0	1.0	12
100	7.5	2.5	0.0	12

Washed in running tap water for 10-15 minutes.

Ethanol 10%, 50%, 70%, 100% - 5 minutes each.

Xylene ethanol (1:1), Xylene I, Xylene II-5 minutes each stained with toluidine blue (1 g 100 ml⁻¹). The slides were mounted in DPX mountant and left undisturbed for 24 hours for observation. Finally slides photographed with an Olympus PM – 10 ADS light microscope.

3.3.5.12. Yield and yield components

The data on the following yield and yield components were recorded employing standard procedures.

Number of flower per plant

Five plants per replication were tagged prior to flowering and the number of flowers produced were recorded daily from commencement of flowering upto harvest and the mean value was worked out and expressed as number of flowers per plant.

Number of pods per plant

The total number of pods produced was counted and the mean was calculated from the selected plants and expressed in numbers.

Fertility co-efficient

The fertility co-efficient was calculated as the ratio of number of matured pods to total number of flowers produced and expressed in percentage.

Number of seeds per pod

The number of seeds per pod was calculated from total number of pods produced by the some plants and their average worked out and expressed in numbers.

Hundred seed weight

The weight of hundred seeds picked at random from replication for every treatment was taken and expressed in gm.

Grain yield per plant

From the same sampling unit yield per plant was recorded and expressed in gm.

3.4. Experiment III : Chemical manipulation for induction of flooding tolerance

An experiment was conducted to study the effect of various plant growth regulators and chemicals for inducing flooding tolerance in the field. The susceptible and tolerant green gram genotypes were used in this experiment. The chemicals and plant growth regulators were tried with different concentrations, sprayed at flowering and pod filling stages. This is done to screen the effective chemicals and their concentrations and also to identify the correct stage of treatment to induce flooding tolerance.

3.4.1. Experimental details

Entries	:	Green gram varieties (CO 4, VBN 1)
Treatments	:	Eight
T1	:	Control
T2	:	Flooding with no spray
T3	:	Gibberellic acid 10 ppm
T4	:	Gibberellic acid 100 ppm
T5	:	Salicylic acid 100 ppm
T6	:	Benzyladenine 10 ppm
T7	:	Benzyladenine 10 ppm
T8	:	Brassinosteroids 0.1 ppm
Design	:	FRBD
Replication	:	3
Plot size	:	4 m x 3 m
Spacing	:	30 cm x 10 cm
Fertilizer	:	25 : 50 N and P kg ha ⁻¹
Date of sowing	:	21.9.2001
Date of harvest	:	VBN1- 18.11.2001 CO 4 - 21.12.2001

Stages of spray

Flowering and Pod filling stages (at termination of flooding treatment)

3.4.2. Field soil characters

The soil characteristic of the experimental site was analysed for the various and chemical characteristics before the start of the experiment and data are presented in Table 2.

3.4.3. Weather conditions

The weekly average maximum and minimum temperature and cumulative rainfall are given in Table 3.

Table 3. Weather data for the cropping period

Standard week	Date	Mean Temperature (°C)		Relative Humidity (%)	Cumulative rainfall (mm)
		Maximum	Minimum		
38	16-22 Sept.	32.9	23.2	87	6.5
39	23-29	30.9	22.2	92	41.5
40	30-6 Oct	31.1	22.4	87	40
41	7-13	31.4	22.7	88	-
42	14-20	31.7	21.7	93	152.3
43	21-27	30.3	19.3	92	130.4
44	28-3 Nov.	30.3	21.7	91	17.6
45	4-10	30.4	22.2	93	30.9
46	11-17	29.6	22.1	91	48.5
47	18-24	28.3	20.9	92	34.1
48	25-1 Dec.	28.7	20.9	91	8.3
49	2-8	28.9	18.0	92	0.5
50	9-15	28.6	15.1	90	-
51	16-22	27.4	19.4	90	14.3
52	23-29	28.0	20.6	90	0.5

3.4.4. Stress imposition

Soil flooding was accomplished by flow of water continuously from an irrigation canal along the field. The soils becoming quickly saturated (within 1 day) with free water remaining on the soil surface for the duration of the experiment. The flood height was maintained at the 3-cm depth for 5 days at flowering and pod filling stages, after which the plots were drained. Control treatments in which the water table was not raised on each plot

3.4.5. Sampling for observation

Five plants from each plot were sampled for recording yields and yield parameters at harvest of each treatment.

3.4.5.1. Yield and yield components

The data on the following yield and yield components were recorded employing standard procedures.

Number of flowers per plant

Ten plants per plot were tagged prior to flowering and the number of flowers produced were recorded daily from commencement of flowering upto harvest and the mean value was worked out and expressed as number of flowers per plant.

Number of pods per cluster

The number of pods produced in each cluster was counted from the same plant and average was worked out and expressed in numbers.

The following yield and yield components were measured as per the procedure described in the experiment II.

- a. Number of pods per plant**
- b. Fertility co-efficient**
- c. Number of seeds per pod**
- d. Hundred seed weight**
- e. Grain yield per plant**

Biological yield (kg ha⁻¹)

The biological yield at each treatment from each growth stages were recorded at harvest and expressed in kg ha⁻¹.

Grain yield (kg ha⁻¹)

Yield per hectare was worked out based on the mean plot yield and expressed in kg ha⁻¹.

Harvest Index (HI)

The HI was calculated as the ratio of seed yield to total dry matter accumulation.

$$HI = \frac{\text{Seed yield}}{\text{TDMA}} \times 100$$

3.4.6. Statistical analysis

Statistical scrutinizing of various data was made employing methods appropriate to the design of experiments and in line with the objective of investigation. The data collected were subjected to statistical analysis as recommended by Panse and Sukhatme (1961).

Results

CHAPTER IV

EXPERIMENTAL RESULTS

The aim of the present investigation was find out primarily to screen the pulses for flooding tolerance and to elucidate information on morphological, physiological, biochemical and molecular mechanism of flooding tolerance. An attempt was also made to manipulate the induction of tolerance with chemicals and plant growth regulators. To achieve the objectives, a series of experiments in laboratory, glass house and field were carried out. The results of various experiments were statistically analysed and the data are presented in appropriate tables with suitable figures.

4.1. Screening of pulses for flooding tolerance under laboratory condition

A preliminary study revealed that the four methods were used for screening pulse genotypes for flooding tolerance. These methods consisted of (1) Triphenyl Tetrazolium Chloride (TTC) reduction method 2) Electrical Conductivity (EC) method, (3) Pressure Chamber (PC) method (xylem water potential of leaves) and (4) root Alcohol Dehydrogenase activity. The results of the experiment are,

4.1.1. Triphenyl Tetrazolium Chloride (TTC) reduction method (Table 4; Fig. 1)

Green gram

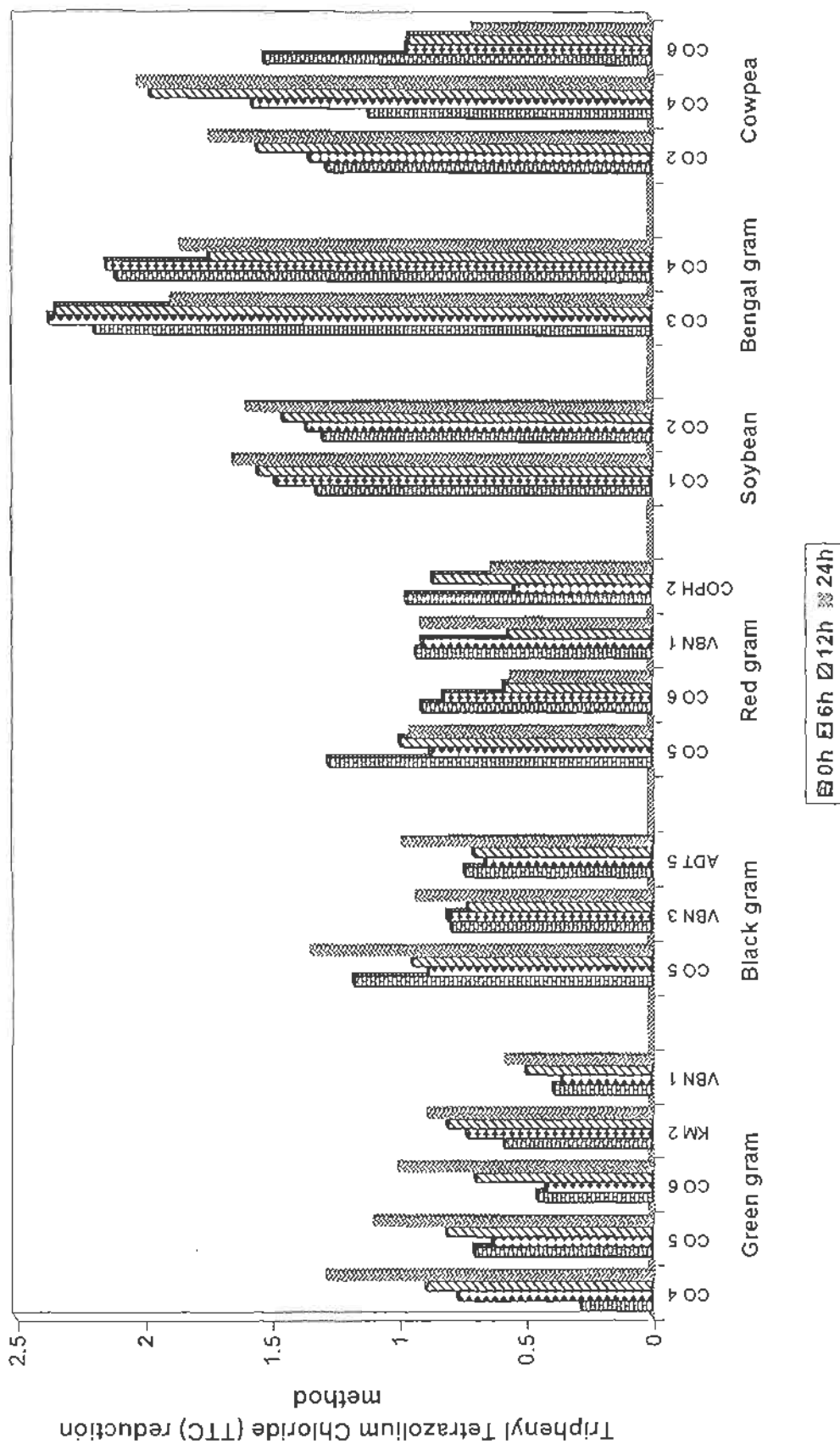
The TTC method was effective in differentiating among the varieties and treatment. Among the varieties CO 4 and CO 5 recorded (0.800 and 0.805) a very high TTC reducing ability compared to VBN 1 which showed a rapid decline in TTC reducing ability comparatively other varieties had lesser TTC reducing ability. Irrespective of the varieties the different duration of flooding like 24 hours recorded high TTC reducing ability followed by 12, 6 and 0 hours. Interaction between varieties and treatments showed that CO 4 (1.280) recorded higher TTC reducing ability compared to VBN 1 (0.573) during flooding for 24 hour. However the CO 5 on par with CO 4 for TTC reducing ability at 24 hour.

Table 4. Screening of pulses for flooding tolerance by Triphenyl Tetrazolium Chloride reduction method, Electrical Conductivity Test, Pressure Chamber Method (Water potential) and Alcohol Dehydrogenase (ADH) Activity

Pulses	Triphenyl Tetrazolium Chloride (TTC) Reduction Method (OD value at 480 nm)					Electrical Conductivity Test (EC)				Water Potential (-MPa)			ADH activity (μ moles NADH oxidised min^{-1} mg^{-1} of sample)			
	0h	6h	12h	24h	Mean	0h	12 h	24 h	Mean	Con.	Flo.	Mean	Con.	Flo.	Mean	
Green gram																
CO 4	0.275	0.761	0.886	1.280	0.800	32.4	47.5	50.0	43.3	0.75	1.05	0.90	0.234	0.933	0.584	
CO 5	0.696	0.625	0.806	1.091	0.805	26.2	68.4	75.6	56.7	0.74	1.23	0.99	0.223	0.729	0.476	
CO 6	0.447	0.414	0.691	0.997	0.637	27.5	59.6	61.9	49.4	0.77	1.33	1.06	0.215	0.398	0.306	
KM 2	0.578	0.726	0.802	0.880	0.745	35.0	64.8	88.0	62.6	0.70	1.27	0.99	0.200	0.426	0.313	
VBN 1	0.382	0.349	0.492	0.573	0.449	26.3	78.3	85.5	63.4	0.92	1.66	1.29	0.265	0.325	0.295	
Mean	0.476	0.575	0.735	0.964	0.688	29.4	63.8	72.0	55.1	0.78	1.31	1.04	0.227	0.562	0.395	
CD P=(0.05)	V 0.058	T 0.052	VT 0.116			V 0.677	T 0.525	VT 1.173			V 0.018	T 0.011	VT 0.026	V 0.006	T 0.003	VT 0.008
Black gram																
CO 5	1.170	0.876	0.940	1.341	1.082	22.4	63.4	77.0	54.3	0.65	1.30	0.99	0.265	0.376	0.330	
VBN 3	0.785	0.802	0.722	0.927	0.809	23.0	66.6	75.0	54.8	0.63	1.36	1.00	0.220	0.368	0.294	
ADT 5	0.731	0.650	0.699	0.980	0.765	25.0	75.1	81.0	61.0	0.65	1.33	0.99	0.296	0.415	0.355	
Mean	0.896	0.776	0.787	1.083	0.885	23.8	68.7	77.5	56.7	0.65	1.27	0.97	0.260	0.386	0.323	
CD P=(0.05)	V 0.034	T 0.040	VT 0.064			V 1.090	T 1.090	VT 1.887			V 0.028	T 0.023	VT 0.040	V 0.003	T 0.002	VT 0.004
Red gram																
CO 5	1.268	0.867	0.985	0.947	1.017	23.3	56.3	72.1	50.6	0.73	1.23	0.98	0.316	0.426	0.371	
CO 6	0.903	0.818	0.578	0.550	0.712	20.7	53.2	74.7	49.5	0.71	1.21	0.96	0.293	0.468	0.380	
VBN 1	0.923	0.901	0.560	0.906	0.823	24.8	55.8	75.6	52.1	0.76	1.06	0.91	0.285	0.486	0.385	
COPH 2	0.962	0.535	0.858	0.629	0.746	21.4	69.2	87.8	57.8	0.81	1.45	0.98	0.281	0.518	0.399	
Mean	1.014	0.780	0.745	0.756	0.824	22.5	57.4	77.5	52.5	0.75	1.24	0.99	0.294	0.474	0.384	
CD P=(0.05)	V 0.023	T 0.023	VT 0.470			V 0.381	T 0.330	VT 0.661			V 0.026	T 0.018	VT 0.037	V 0.003	T 0.002	VT 0.004
Soybean																
CO 1	1.317	1.476	1.544	1.642	1.495	22.2	67.4	73.7	54.4	0.86	1.75	1.30	0.218	0.644	0.431	
CO 2	1.290	1.355	1.446	1.592	1.421	38.9	65.9	68.8	57.9	0.81	1.85	1.33	0.241	0.722	0.481	
Mean	1.304	1.416	1.495	1.617	1.458	30.5	66.7	71.2	56.2	0.84	1.80	1.32	0.230	0.683	0.456	
CD P=(0.05)	V 0.044	T 0.063	VT 0.089			V 14.869	T 18.211	VT 25.755			V 0.012	T 0.012	VT 0.017	V 0.004	T 0.002	VT 0.006
Bengal gram																
CO 3	2.184	2.364	2.336	1.886	2.193	23.4	48.3	87.0	52.9	0.75	1.33	1.04	0.332	0.496	0.414	
CO 4	2.104	2.14	1.736	1.850	1.451	30.5	55.8	79.8	55.3	0.73	1.25	0.99	0.275	0.344	0.345	
Mean	2.144	2.239	2.036	1.868	2.072	27.0	52.1	83.4	54.1	0.74	1.29	1.01	0.314	0.495	0.374	
CD P=(0.05)	V 0.159	T 0.225	VT 0.318			V 9.847	T 12.060	VT 17.055			V 0.018	T 0.018	VT 0.025	V 0.004	T 0.004	VT 0.005
Cowpea																
CO 2	1.274	1.343	1.548	1.738	1.476	16.4	40.7	45.0	34.0	0.97	2.03	1.50	0.303	0.888	0.734	
CO 4	1.110	1.568	1.969	2.021	1.667	17.2	38.4	54.9	36.8	0.81	1.43	1.12	0.28	0.578	0.429	
CO 6	1.524	0.964	0.956	0.706	1.038	18.0	38.8	52.1	36.3	0.83	1.18	1.32	0.295	0.756	0.525	
Mean	1.303	1.291	1.491	1.489	1.394	17.2	39.3	50.6	35.7	0.87	1.75	1.31	0.293	0.734	0.516	
CD P=(0.05)	V 0.206	T 0.238	VT 0.413			V 0.401	T 0.401	VT 0.695			V 0.004	T 0.003	VT 0.005	V 0.003	T 0.002	VT 0.004

Con. - Control ; Flo. - Flooding for 5 days

Fig. 1. Screening of pulses for flooding tolerance by Triphenyl Tetrazolium Chloride (TTC) reduction method (OD value at 480 nm)



Black gram

Significant differences were found between the varieties. The mean of TTC reducing ability was high in CO 5 (1.082) compared to VBN3 (0.809) and ADT 5 (0.765). Irrespective of the varieties, the treatment 24 hours flooding showed higher TTC reducing ability followed by 0, 12 and 6 hours. The variety CO 5 recorded higher TTC reducing ability compared to other varieties at 24 hours flooding .

Red gram

The TTC reducing ability was significantly different with varieties. The mean of TTC was high in CO 5 compared to other varieties like CO 6, VBN 1 and COPH 2. Among the different duration of submersion of roots, 24 hours recorded higher TTC reducing ability compared to other hours like 12, 6 and 0 (control). The all varieties had decrease in TTC reducing ability by increased soaking duration of roots.

Soybean

There was significant variation in the TTC reducing ability of varieties under different duration of submersion in water. The variety CO 1 (1.495) recorded higher TTC reducing ability than CO 2 (1.421). Irrespective of the varieties, the treatment 24 hours submersion increases the reducing ability compared to 0 hours and followed by 12 and 6 hours. Interaction between varieties and treatments were nonsignificant.

Bengal gram

Significant differences were exists between the varieties. The variety CO 3 (2.193) had high TTC reducing ability than CO 4 (1.951). Among the treatments, 24 hours submersion decreases the reducing ability of TTC compared to 12 and 6 hours over to control. Interaction between the varieties and treatment non-significant.

Cowpea

There was a significant difference between the varieties and treatments. The variety CO 4 (1.667) recorded high TTC reducing ability compared to CO 6 (1.476) and CO 2 (1.038). However CO 2 showed a decline in TTC reducing ability. The treatments 12 and 24 hours recorded higher TTC reducing ability compared to 6 hours and control (0 hours). Interaction between varieties and treatments showed 24 hours

soaking increases the reducing ability of TTC in CO 4 (2.021) compared to CO 6 (1.738). However, the CO 2 had decrease in TTC reducing ability at 24 hours' soaking.

4.1.2. Electrical Conductivity Test (Percentage) (Table 4; Fig. 2)

Green gram

A conductivity method measuring ion efflux due to flood injury has been used to evaluate the relative tolerance of plants. The leakage of solutes by roots varied significantly between the varieties. The variety VBN 1 (63.4) had higher amount of the cell solutes leached from the roots compared to CO 4 (43.3). However other varieties recorded high ion efflux like KM 2 (62.6), CO 5 (56.7) and CO 6 (49.4). Among the treatment 24 hour soaking recorded higher leakage of solutes compared to 12 and 0 hours. Interaction between varieties and treatment showed the VBN 1 (78.3) had higher leakage of solutes compared to CO 4 (41.5) at 12 hour flooding.

Black gram

There was no significant difference between the varieties for leakage of solutes or ions. Irrespective of the varieties flooding for 12 hours decreases the ion leakage in roots compared to 24 hours flooding. Interaction between varieties and treatments indicated that ADT 5 (81) showed higher amount of leakage followed by CO 5 (77) VBN3 (75) at 24 hours flooding.

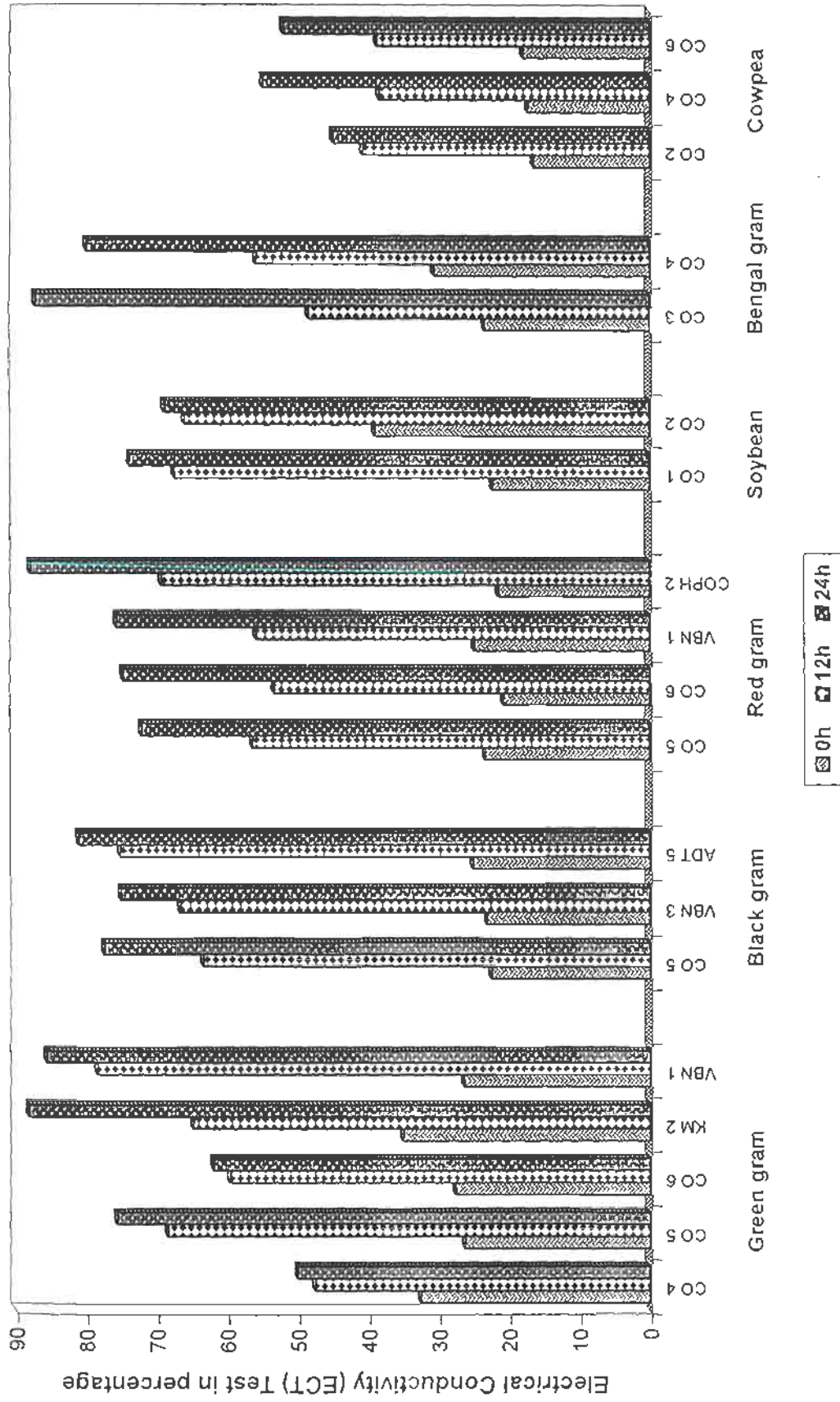
Red gram

There was less significant difference between the varieties. The variety VBN 1 (52.9) recorded higher amount of solute leakage compared to CO 5 (51) and CO 6 (50). Among the treatments, 24 hours flooding increases the ion leakage compared to 0 and 12 hours. In the varieties VBN 1 and CO 6 showed slightly higher amount of leakage compared to CO 5 at 24 hours flooding.

Soybean

The solute leakage (ion efflux) of roots was significantly increased under flooded conditions. There was no significant difference between the varieties. However the variety CO 2 recorded higher amount of ion efflux than CO 1. Among

Fig. 2. Screening of pulses for flooding tolerance by Electrical Conductivity (EC) Test (in %)



the treatments, 24 hours soaking increases the ion efflux than 12 hours and 0 hours soaking. Interaction between varieties and treatments were nonsignificant.

Bengal gram

Similar to soybean there was no significant difference between the varieties. The variety CO 4 (55) recorded higher amount of ion leakage than CO 3 (53). The treatment 24 hours flooding increases the leakage of solutes compared to 0 and 12 hours flooding. Interaction between varieties and treatments showed the CO 3 recorded higher ion efflux than CO 4 at 24 hours flooding over control.

Cowpea

There was significant difference between the varieties for ion leakage. The variety CO 2 (36.8) recorded higher amount of ion leakage than CO 6 (36.3) and CO 4 (34.0). Among the treatments, 24 hours flooding increases the ion leakage compared to 12 hours and over to control. Interaction between varieties and treatments showed that CO 2 (45) recorded lower amount of leakage than to CO 4 (52) and CO 6 (55) at 24 hours soaking.

**4.1.3. Pressure Chamber Method (Leaf xylem water potential) (-MPa)
(Table 4; Fig. 3)**

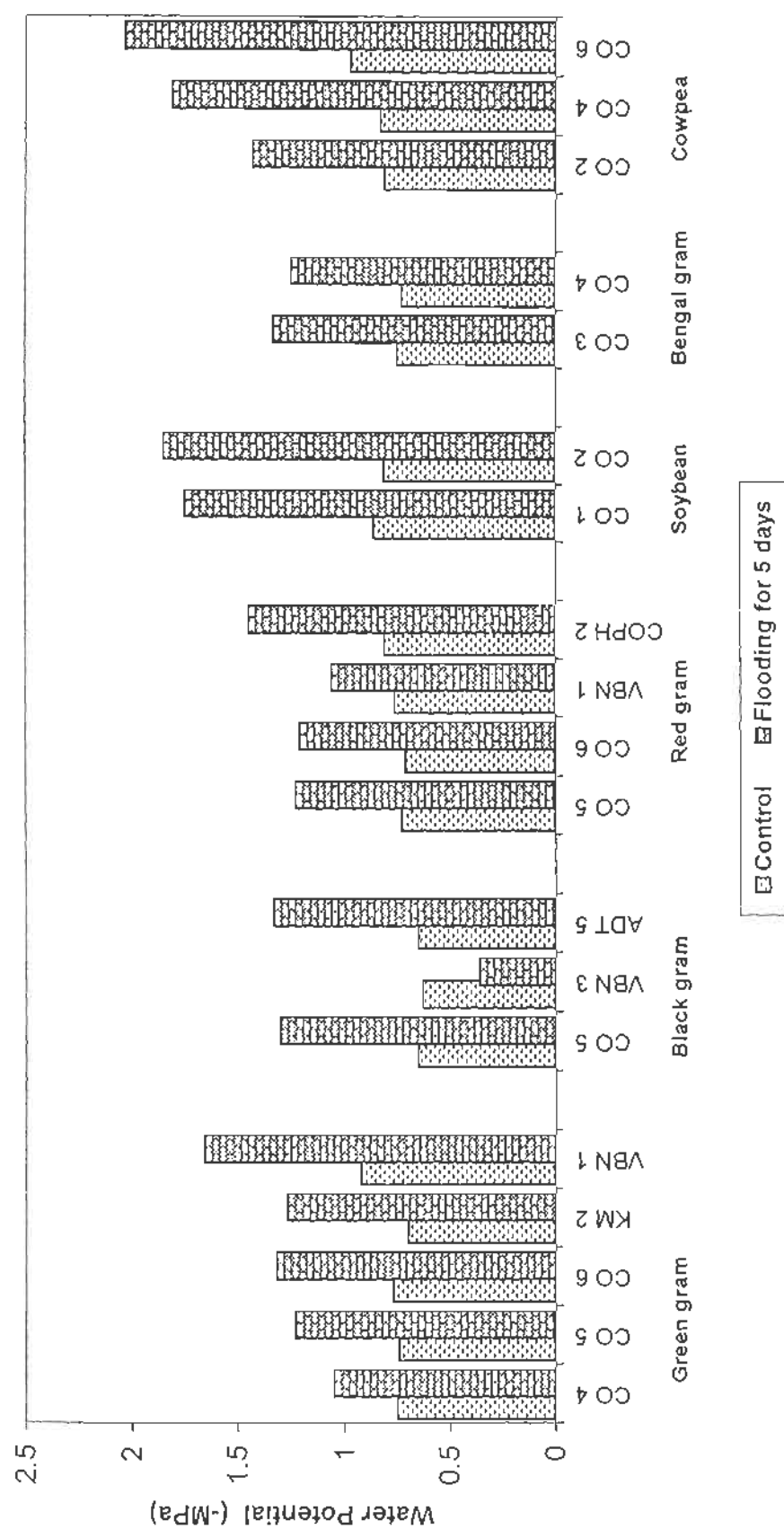
Green gram

Leaf xylem water potential provided good differentiation among the varieties. The variety CO 4 (-0.90) recorded decreasing water potential compared to VBN 1 (-1.29). However other varieties like CO 5, CO 6 and KM 2 showed lower water potential than CO 4 varieties. Among the treatments, flooding decreases the water potential (-1.31) compared to control (- 0.78). Interaction between varieties and treatments showed that CO 4 (-1.27) had increased water potential compared to VBN 1 (-1.66) under flooded conditions.

Black gram

There was no significant difference between the varieties. Decreasing water potential indicates flooding tolerance. The treatment flooding decreased the water potential (-1.23) compared to control (-0.73). The all varieties had similar decrease

Fig. 3. Screening of pulses for flooding tolerance by Water Potential (-MPa)



trend in the water potentials. However, the VBN 3 (-1.36) had higher decrease in water potential compared to CO 5 (-1.30) and ADT 5 (-1.33).

Red gram

There was less significant difference between the varieties. The variety CO 5 and COPH 2 was on par with each other for water potentials. Flooding treatments decreases the water potential (-1.75) compared to control (-0.75). The COPH 2 recorded lesser water potential (-1.45) than CO 5, CO 6 and VBN 1 under flooded situations.

Soybean

No significant difference between the varieties. The both varieties CO 1 and CO 2 recorded water potentials on par with each other. Similar to other crops it also decreases the water potential in flooded treatments. The variety CO 2 (-1.85) recorded lesser water potential compared to CO 1 (-1.75) under flooding condition.

Bengal gram

As like that of soybean, no significant difference between the varieties. Both the varieties CO 3 and CO 4 recorded lower water potentials. Flooding decreases the water potential compared to control. The variety CO 3 (-1.33) recorded lower water potential compared to CO 4 (-1.25) in flooded situations.

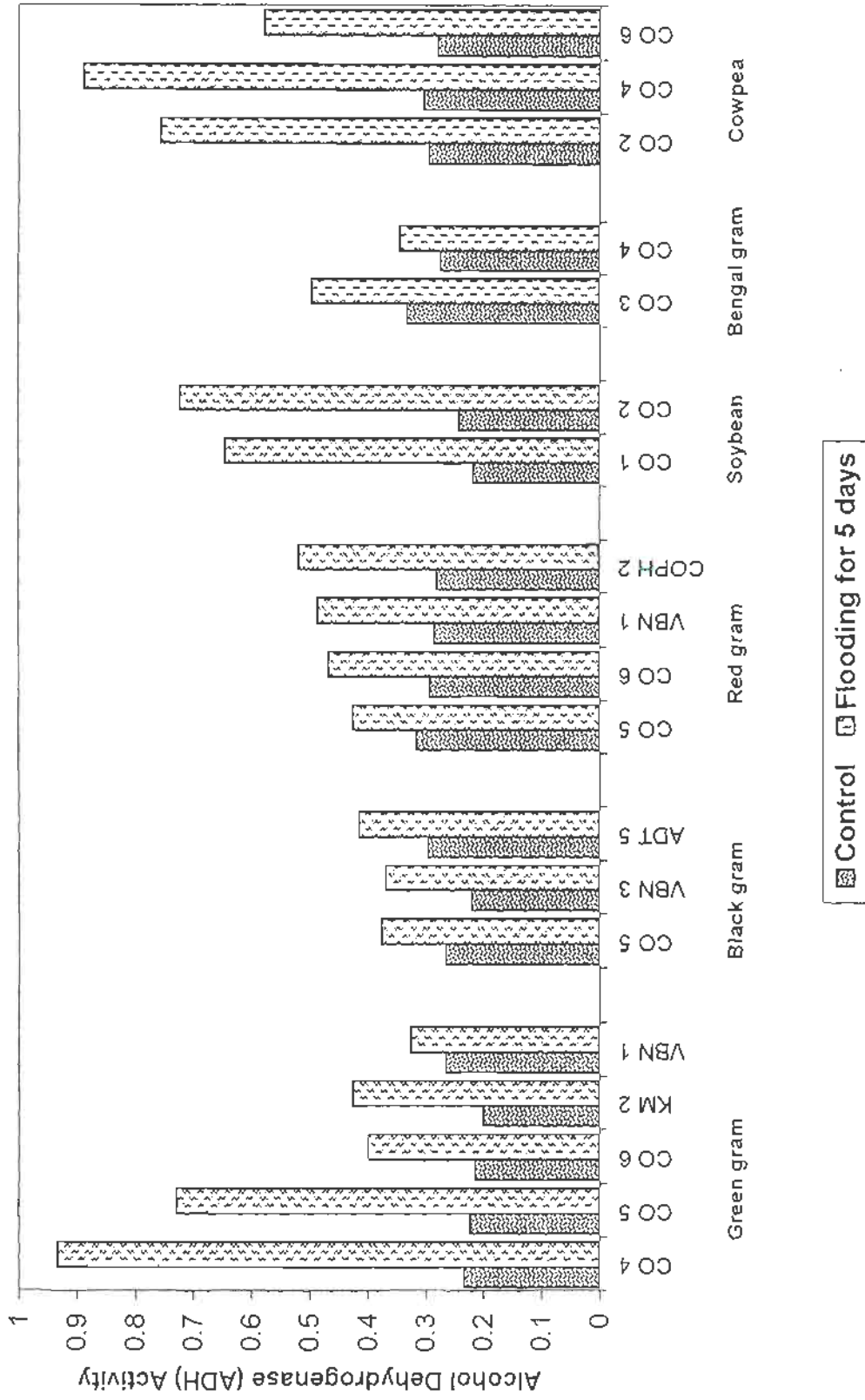
Cowpea

Significant difference was found between the varieties for leaf xylem water potentials. Among the varieties CO 4 showed higher water potentials (-1.12) compared to CO 6 (-1.32) and CO 2 (-1.50). The flooding stress decreases water potentials compared to control. The variety CO 4 (-1.43) recorded higher water potential than CO 2 (-2.03) by flooding treatments.

4.1.4. Alcohol Dehydrogenase (ADH) activity (Table 4; Fig. 4)

Flooding significantly increases the ADH activity compared to control in all pulse crops.

Fig. 4. Screening of pulses for flooding tolerance by Alcohol Dehydrogenase (ADH) Activity (μ moles NADH oxidised $\text{min}^{-1} \text{mg}^{-1}$ of sample)



Green gram

Significant difference found between the varieties for their ADH activity. The variety CO 4 showed higher activity in ADH (0.584) and lower in VBN 1 (0.295). However the varieties like CO 5, KM 2 and CO 6 recorded lower activity of ADH compared to CO 4. Flooding increases the ADH activity (0.562) compared to control (0.227). The variety CO 4 had higher activity of ADH (0.933) followed by CO 5 (0.729), KM 2 (0.426), CO 6 (0.398) and VBN 1 (0.325) during flooded conditions.

Black gram

There was less difference between the varieties. The variety ADT 5 recorded higher activity of ADH (0.355) followed by CO 5 (0.330), VBN 3 (0.294). Flooding increases the ADH activity. Comparatively ADT 5 showed higher activity of ADH (0.415) during flooded conditions compared to control (0.296).

Red gram

Similar to black gram, here also less differences between the varieties for ADH activity. The variety VBN 1 showed higher activity (0.385) compared to CO 6 (0.380) and CO 5 (0.371). Flooding increases ADH activity compared to control. However higher activity of ADH was noticed in VBN 1 (0.486).

Soybean

There was significant difference between the varieties. The variety CO 2 recorded higher mean activity of ADH (0.481) compared to CO 1 (0.431). Flooding increases ADH activity (0.683) compared to control (0.230). The variety CO 2 showed marked increase in ADH activity (0.722) compared to CO 1 (0.644) under flooded treatments.

Bengal gram

The ADH activity did not change very much under flooded conditions in both genotypes. The mean of ADH activity was higher in CO 3 (0.414) than CO 4 (0.345). Flooding increases the ADH activity (0.445) compared to control (0.314). The variety Co 3 recorded higher activity of ADH under flooding conditions compared to control.

Cowpea

There was significant difference exists between the varieties. The variety CO 4 recorded higher activity of ADH (0.734) compared to CO 6 (0.525) and CO 2 (0.429). Flooding significantly increases the ADH activity (0.734) compared to control (0.293). The variety CO 4 showed higher activity of ADH (0.888) compared to CO 2 (0.518) under flood condition. However the CO 6 recorded higher activity of ADH next to CO 4.

4.2. Glass House Study

Based on the laboratory screening methods, the screened tolerant and susceptible pulse genotypes used in the pot culture experiment. Various morpho-physiological, biochemical, molecular mechanism and yield components were studied and subjected to statistical analysis. The results obtained are presented in this section.

4.2.1. Morphological characters

4.2.1.1 Plant height (cm) (Table 5)

Cowpea

The data on plant height was recorded at vegetative, flowering and pod filling stages. Flooding treatments decreased the plant height. Among the genotypes used, the CO 2 had maximum reduction in plant height (25%) compared to CO 4 (16%) at vegetative stage due to 10 days of flooding. The decrease in plant height was maximum at vegetative stage as compared to flowering and pod filling stages. The longer period of flooding (10 days) treatment had detrimental effect on plant height showed maximum reduction was 20.1% as compared to control at vegetative stage. At pod filling stage, flood stress seems to be had lesser effect on plant height (11.6%). Interaction between varieties and stages was significantly showed that CO 4 recorded higher plant height than CO 2 at pod filling stage.

Soybean

The mean plant height was observed significantly highest in the genotype CO 1 (33.5) and lowest in CO 2 (25.2). There was less difference reduction in plant

Table 5. Effect of flood stress on plant height (cm) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	16.1	14.8	13.5	14.8	27.7	25.8	23.9	25.8	48.4	44.8	43.4	45.6	28.7
	CO 2	14.8	12.6	11.1	12.8	25.1	23.4	19.9	22.8	36.2	32.8	31.4	33.5	23.0
	Mean	15.4	13.7	12.3	13.8	26.4	24.6	21.9	23.9	42.3	38.8	37.4	39.4	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		2.142	2.623	2.623		3.710	4.544	3.710		6.426				
Soybean	CO 1	17.9	16.3	16.1	16.8	31.0	29.4	28.4	29.6	56.2	53.7	52.2	54.0	33.5
	CO 2	16.8	16.1	15.6	16.4	23.8	22.0	21.6	22.5	39.2	35.6	35.5	36.8	25.2
	Mean	17.4	16.2	15.8	16.6	27.4	25.7	25.0	26.0	47.7	44.7	43.9	45.4	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		2.045	2.504	2.504		3.542	4.338	3.543		6.135				
Green gram	CO 4	22.7	21.4	18.3	20.8	45.7	43.1	42.2	43.7	77.5	74.0	72.2	74.6	46.3
	VBN 1	17.5	17.0	14.8	16.4	24.6	22.2	20.1	22.3	40.8	38.5	36.0	38.4	25.7
	Mean	20.1	19.3	16.5	18.7	35.1	32.6	31.2	33.0	59.1	56.3	54.1	56.5	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		1.515	1.857	1.857		2.626	3.216	2.626		4.548				

height due to flooding at vegetative stage. No significant difference between the treatments. Maximum reduction of plant height (9.2%) was recorded in 10 days of flooding at vegetative stage compared to pod filling stage (8%). At different stages, pod filling showed higher plant height than other growth stages. Significant interaction between varieties and stages revealed that plant height of CO 2 was lesser (2.4%) than CO 1 at vegetative stage. Other interactions were non significant.

Green gram

The mean plant height recorded more in CO 4 (46.3) as compared to VBN 1 (25.7). Among the treatments flooding of 5 days showed less reduction in plant height at vegetative and pods filling stages. Maximum reduction of plant height observed in 10 days of flooding at vegetative stage (17.9%) compared to flowering stage (12.5%). At flowering stage, the 10 days of flooding recorded lesser plant height in VBN 1 compared to CO 4 over control. Interaction between genotypes and stages found that genotype VBN 1 had lesser plant height (22.3) as compared to CO 4 (43.7) at flowering stage.

4.2.1.2. Root length (cm) (Table 6; Fig. 5)

Cowpea

Flooding increases the root length in both varieties significantly. The mean value of root length was higher in CO 4 (17.2) than CO 2 (14.2). Among the treatments, flooding for 10 days at vegetative stage recorded higher root length (75.8 %) compared to 5 days of flooding. Irrespective of the varieties, the treatment flooding for 10 days was recorded higher root length in all growth stages. At pod filling stages, the flooding treatment increases root length (19.1) compared to vegetative (16.1) and flowering stage (12.7). Interaction effects were non-significant.

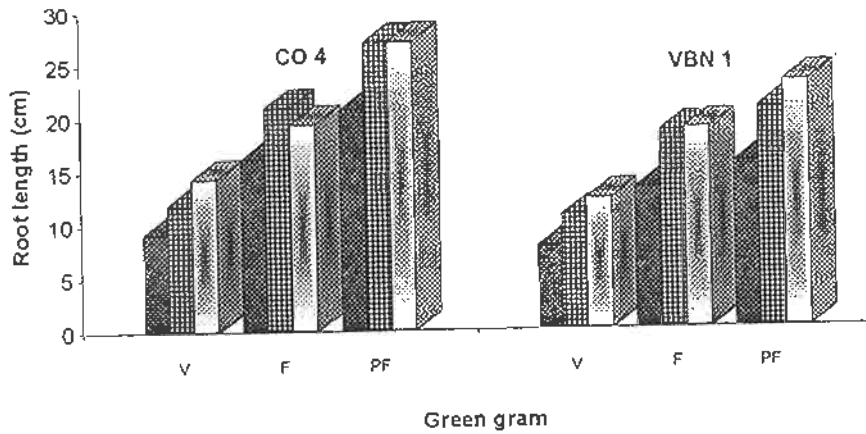
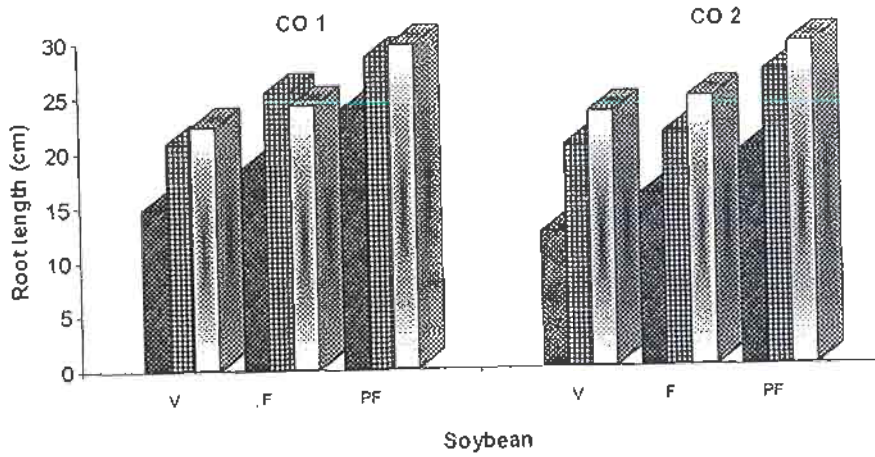
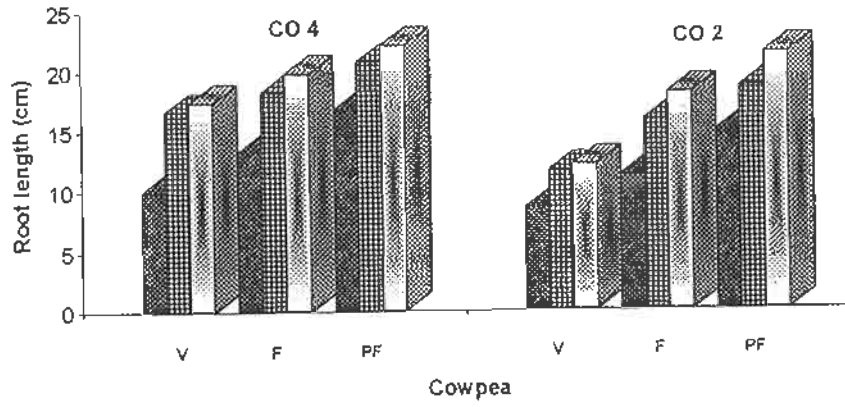
Soybean

The response of root length to flood stress was different among the varieties. Among the varieties, the highest root length was noticed in CO 1 (23.1) as against the lowest in CO 2 (21.6) and the difference was found to be significant. Flooding for 10 days increases the root length in CO 2 to (88.7%) compared to CO 1 (50.7%) at

Table 6. Effect of flood stress on root length (cm) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Flooding for 10 days			Flooding for 10 days			Flooding for 10 days							
	Control	5 days	10 days	Control	5 days	10 days	Control	5 days	10 days					
Cowpea	CO 4	9.9	16.6	17.4	14.6	13.2	18.2	19.8	17.1	16.8	20.8	22.1	19.1	17.2
	CO 2	8.5	11.6	12.1	10.7	11.2	15.9	18.1	15.1	14.9	18.6	21.4	18.3	14.2
	Mean	9.2	14.1	14.7	12.7	12.2	17.1	18.9	16.1	15.8	19.7	21.7	19.1	
CD (P=0.05)		V	T	S		VXT	TX S	VXS		VXTXS				
		1.114	1.364	1.364		1.929	2.363	1.929		3.342				
Soybean	CO 1	14.8	20.8	22.3	19.3	18.5	25.4	24.2	22.7	23.8	28.6	29.7	27.4	23.1
	CO 2	12.4	20.4	23.4	18.7	15.8	21.4	24.7	20.6	19.7	27.0	29.6	25.4	21.6
	Mean	13.6	20.6	22.9	19.0	17.2	23.4	24.5	21.7	21.8	27.8	29.6	26.4	
CD (P=0.05)		V	T	S		VXT	TX S	VXS		VXTXS				
		1.114	1.364	1.364		1.930	2.363	1.930		3.342				
Green gram	CO 4	9.2	11.9	14.3	11.8	16.1	21.0	19.4	18.8	20.7	27.0	27.1	24.9	18.5
	VBN 1	7.8	10.7	12.2	10.2	13.1	18.5	18.7	16.8	15.4	20.7	23.1	19.7	15.6
	Mean	8.5	11.3	13.2	11.0	14.6	19.8	19.0	17.8	18.1	23.9	25.1	22.3	
CD (P=0.05)		V	T	S		VXT	TX S	VXS		VXTXS				
		1.124	1.377	1.377		1.947	2.385	1.947		3.373				

Fig. 5. Effect of flood stress on Root length (cm)



■ 0 days ▨ 5 days ▩ 10 days

V - Vegetative; F - Flowering; PF - Pod Filling

vegetative stage. At flowering stage 10 days of flooding increases root length about 70.2% as compared to pod filling stages (35.8%). Interaction between varieties and treatments was found to be significant.

Green gram

Significant difference in mean root length were observed among the varieties, with the highest being recorded in CO 4 (18.5 cm) and lowest in (15.6). Among the flooding treatments flooding for 10 days increases root length compared to 5 days flooding at all stages. At different stages, pod-filling stage recorded higher root length followed by flowering and vegetative stages. Interaction between variety and stage revealed that CO 4 recorded higher root length (24.9) compared to VBN 1 (19.7) at pod filling stages.

4.2.1.3. Root numbers (Table 7)

Cowpea

There is no significant difference in mean root numbers for both variety CO 4 (18.4) and CO 2 (17.8). Flooding for 10 days increases root numbers in both varieties CO 4 and CO 2 at all growth stages. However, the longer duration of flooding (10 days) recorded higher root numbers in CO 4 (77.8%) compared to CO 2 (61.3%) over control at vegetative stage. At vegetative stage 10 days flooding recorded significantly higher root numbers (68.5%) compared to lower root number (35.9%) at pod filling stage. Flooding for 5 days recorded lower root numbers compared to 10 days of flooding at all growth stages. Interaction effects were non-significant.

Soybean

Similar to Cowpea, no significant difference in mean root numbers for both varieties. Among the varieties, the CO 4 recorded higher per cent root numbers (81.8) compared to VBN 1 had (46.1) in 10 days of flooding at vegetative stage. The flooding of 10 days recorded higher 62.5 per cent of root numbers at vegetative stage compared to flowering (39.5) and pod filling stages (42.9). Among the stages, the vegetative stage recorded higher number of roots compared to pod filling and flowering stages. Interaction effect was found to be non significant.

Green gram

The number of roots increases steadily upto pod filling stages. Flooding significantly increases the root numbers in both varieties (CO 4 and VBN 1). The variety CO 4 significantly increases the number of roots 75% over control as compared to VBN 1 (57%) by 10 days of flooding at vegetative stages. Among the treatment 10 days of flooding increases root numbers (25.7) compared to flooding for 5 days (22.7) over control (17.8). At different stages, pod filling recorded significantly more root numbers compared to flowering and vegetative stages. Interaction effects were non-significant.

4.2.2. Nodule status of pulses

4.2.2.1. Nodule number per plant (Table 8; Fig. 6)

Cowpea

The nodule number per plant increased upto flowering after which there was a marked decline. There was no significant difference in mean nodule number in both varieties (CO 2 and CO 4). Among flooding treatments, 10 days of flooding recorded higher reduction of nodule number (35.2%) compared to 5 days flooding (18.2%). At different stages the pod filling stage recorded less number of nodules (4.7) compared to vegetative (7.2) and flowering (9.8) stages. Irrespective of the treatments, the variety CO 4 had more reduction in nodule number (41.4%) as compared to CO 2 (30.8%) at vegetative stage. Interaction effects were found to be non-significant.

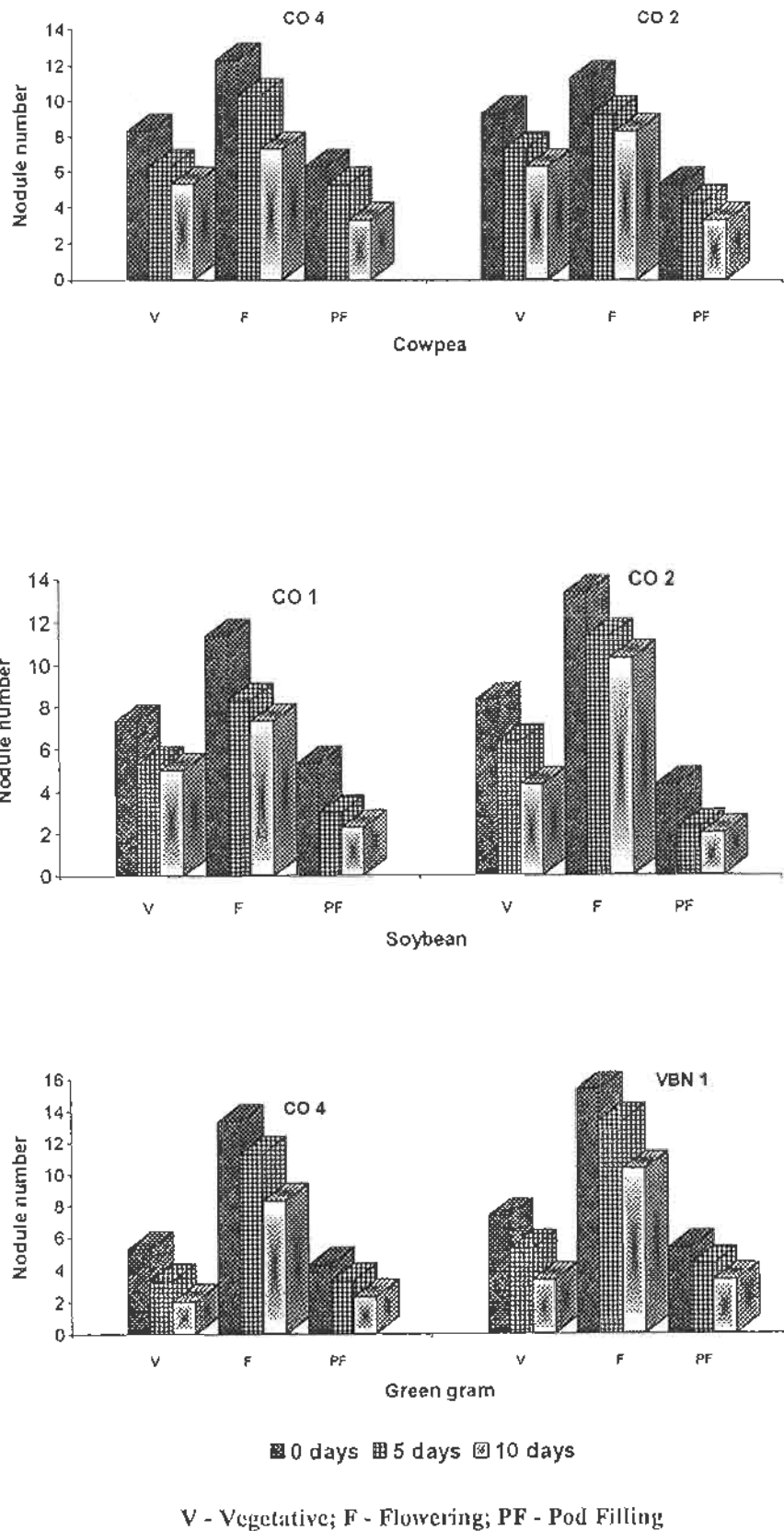
Soybean

The mean nodule number per plant was significantly different in both varieties (CO 1 and CO 2). The variety CO 2 recorded significantly more reduction in nodule number per plant compared to CO 1 on 10 days of flooding at vegetative stage. However, the variety CO 1 had more reduction in nodule number (36.6%) compared to CO 2 (46.5%) on 10 days of flooding at pod filling stages. Among the flooding treatments, 10 days of flooding recorded more reduction in nodule number per plant at pod filling stage compared to 5 days of flooding at flowering stage. Among the stages

Table 8. Effect of flood stress on nodule number per plant in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean			
	Control	Flooding for 10 days		Control	Flooding for 10 days		Control	Flooding for 10 days					
		5 days	Mean		5 days	Mean		5 days	Mean				
Cowpea	8.3	6.3	5.3	6.7	12.3	10.3	7.3	10.0	6.3	5.3	3.3	5.0	7.2
CO 2	9.3	7.3	6.3	7.7	11.3	9.3	8.3	9.7	5.3	4.3	3.3	4.3	7.2
Mean	8.8	6.8	5.8	7.2	11.8	9.8	7.8	9.8	5.8	4.8	3.3	4.7	
CD (P=0.05)	V	T	S		VXT	TX S	VXS		VXTXS				
	0.843	1.032	1.032		1.460	1.788	1.460		2.529				
Soybean	7.3	5.3	5.0	5.9	11.3	8.3	7.3	9.0	5.3	3.0	2.3	3.6	6.14
CO 2	8.3	6.3	4.3	6.3	13.3	11.3	10.3	11.6	4.3	2.3	2.0	2.9	6.96
Mean	7.8	5.8	4.7	6.1	12.3	9.8	8.8	10.3	4.8	2.7	2.2	3.2	
CD (P=0.05)	V	T	S		VXT	TX S	VXS		VXTXS				
	0.822	1.007	1.007		1.425	1.745	1.425		2.468				
Green gram	5.3	3.3	2.0	3.6	13.3	11.3	8.3	11.0	4.3	3.3	2.3	3.3	5.96
CO 4	7.3	5.3	3.3	5.3	15.3	13.3	10.3	13.0	5.3	4.3	3.3	4.3	7.55
VBN 1	6.3	4.3	2.7	5.7	14.3	12.3	9.3	12.0	4.8	3.8	2.8	3.8	
CD (P=0.05)	V	T	S		VXT	TX S	VXS		VXTXS				
	0.829	1.016	1.016		1.437	1.760	1.437		2.489				

Fig. 6. Effect of flood stress on Nodule number per plant



pod-filling stage recorded less number of nodules per plant compared to other stages. Interaction between varieties and stages was found to be significant.

Green gram

Significant differences in nodule number were noticed in both varieties (CO 4 and VBN 1). Among the varieties, the VBN 1 (7.55) recorded more number of nodules compared to CO 4 (5.96). The flooding treatment significantly decreases nodule number in both varieties (CO 4 and VBN 1). Flooding for 10 days decreases the nodule number (2.0) compared to control (5.3) in CO 4 at vegetative stages. However, highest reduction of nodule number was noticed in CO 4 (62.3%) compared to VBN 1 (54.8%) on 10 days of flooding at vegetative stages. Among the treatment, flooding for 10 days noticed higher reduction of nodule number compared to 5 days of flooding. Among the stages, the pod filling stage recorded less number of nodules followed by vegetative and flowering stages. Interaction effects were nonsignificant.

4.2.2.2. Nodule dry weight per plant (mg) (Table 9)

Cowpea

The nodule dry weight per plant increased upto flowering stage (135.6), thereafter it declined. Among the varieties used the CO 2 had maximum mean dry weight of nodule compared to CO 4. Flooding treatment decreases nodule dry weight in both varieties. The variety CO 4 recorded lesser dry weight of nodule compared to CO 2 on 10 days of flooding over control at all growth stages. Maximum reduction of dry weight of nodule noticed in CO 2 (35.27) on 10 days of flooding compared to control at pod filling stages. The flooding for 10 days recorded more reduction in mean nodule dry weight per plant compared to 5 days of flooding. However, the maximum reduction of nodule dry weight (31.1%) was noticed by 10 days flooding at vegetative stage. Among the stages, pod filling observed that less dry weight of nodules (66.4 mg) per plant compared to vegetative (75.4) and pod filling (135.6) stages.

Table 9. Effect of flood stress on nodule dry weight (mg. plant⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	85.3	67.2	60.0	70.8	145.0	133.4	121.5	133.3	73.3	63.8	62.9	66.7	90.3
	CO 2	97.7	74.8	65.9	79.5	157.5	130.1	126.4	133.1	83.3	61.4	54.0	66.2	94.5
	Mean	91.5	70.9	63.0	75.4	151.3	131.8	124.0	135.6	78.3	62.6	58.5	66.4	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		2.004	2.454	2.454		3.471	4.251	3.471		6.012				
Soybean	CO 1	79.1	60.8	52.9	64.3	115.3	94.6	79.1	96.3	57.6	53.0	49.1	53.2	71.3
	CO 2	84.3	64.9	58.1	69.1	122.3	107.8	93.2	106.4	63.4	48.1	41.0	50.9	75.5
	Mean	81.7	62.9	55.5	66.7	118.8	99.2	86.2	101.4	60.5	50.5	45.1	52.0	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		2.109	2.583	2.583		3.653	4.474	3.653		6.328				
Green gram	CO 4	74.2	56.2	59.1	63.2	112.3	100.9	79.0	97.4	64.3	61.3	49.0	58.2	72.9
	VBN 1	82.1	67.6	62.0	70.6	121.4	97.8	77.1	98.7	60.9	55.8	51.8	56.2	75.2
	Mean	78.6	61.9	60.6	66.9	116.8	99.3	78.1	98.1	60.9	58.6	51.8	57.2	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		3.116	3.817	3.817		5.398	6.611	5.398		9.349				

Soybean

The nodule dry weight per plant was significantly differed among the varieties. The variety CO 2 (75.5) recorded highest overall mean nodule dry weight compared to CO 1 (71.3). Under flooding treatments 10 days flooding noticed higher reduction of nodule dry weight (32.1%) compared to 5 days of flooding (23%) at vegetative stage. Among the stages, the pod filling stage recorded lesser dry weight of nodules followed by vegetative and pod filling stages. Interaction between treatments and stages revealed that 10 days flooding decreases nodule dry weight (32%) compared to 5 days flooding (23%) at vegetative stage. Interaction effects between V and S, V and T and S were also found to be significant.

Green gram

Nodule dry weight per plant was not significantly differed in both the varieties (CO 4 and VBN 1). Flooding treatment decreases the nodule dry weight per plant in both varieties at all the growth stages. Among the flood treatments, flooding for 10 days (33.1%) recorded more reduction of nodule dry weight compared to 5 days of flooding (15%) over control at flowering stage. For growth stages, the pod filling stage showed lesser dry weight of nodules followed by vegetative and flowering stages. Interaction between treatments and stages were significantly shown that flooding for 10 days decreases nodule dry weight was 33 per cent compared to 5 days flooding (15%) at flowering stage.

4.2.2.3. Leghaemoglobin (mg g^{-1} dry nodule) (Table 10; Fig. 7)

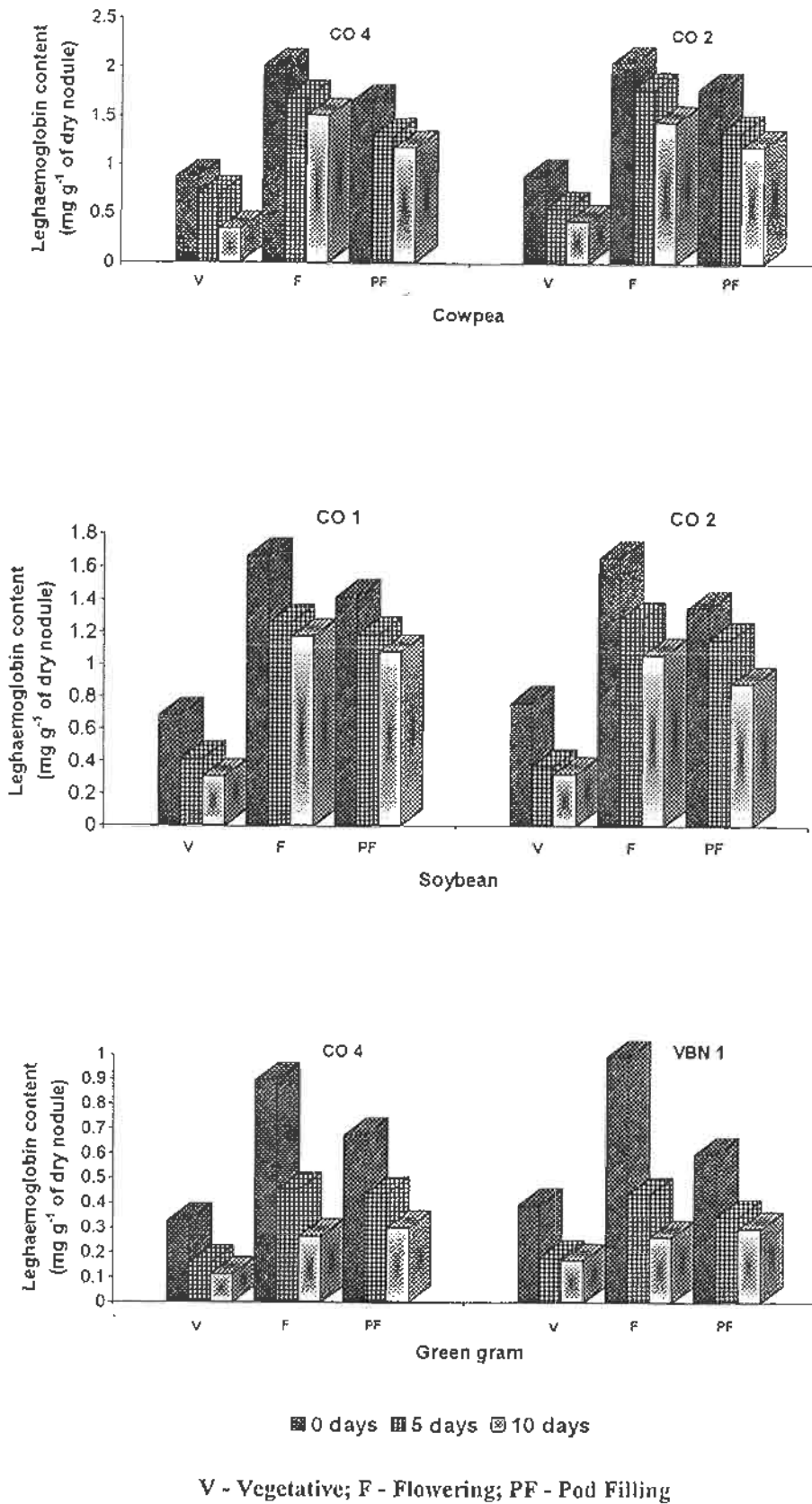
Cowpea

The leghaemoglobin content showed its peak upto flowering stage. There is no significant difference between the varieties. Flooding treatments reduces the leghaemoglobin content at all the growth stages. The variety CO 4 showed higher reduction of leghaemoglobin content (61.1%) as compared to CO 2 (52.4%) under 10 days of flooding at vegetative stage. Among the treatments flooding for 10 days recorded higher reduction of leghaemoglobin content (56.8%) compared to 5 days of flooding (28.6) at vegetative stage. Regarding growth stages, vegetative stage showed

Table 10. Effect of flood stress on leghaemoglobin content (mg. g⁻¹ of dry nodule) in pulses at different growth stages

Pulses	Vegetative stage				Flowering stage				Pod filling stage				Genotypes Mean	
	Control		Flooding for 10 days		Control		Flooding for 10 days		Control		Flooding for 10 days			
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days		
Cowpea	CO 4	0.876	0.703	0.340	0.640	2.017	1.693	1.517	1.742	1.660	1.313	1.186	1.387	1.256
	CO 2	0.896	0.563	0.426	0.629	2.056	1.793	1.457	1.769	1.793	1.363	1.207	1.454	1.284
	Mean	0.887	0.633	0.383	0.634	2.037	1.743	1.427	1.756	1.727	1.338	1.197	1.420	
CD (P=0.05)		V	T	S		VXT	TXS	VXS		VXTXS				
		0.108	0.133	0.133		0.188	0.230	0.188		0.326				
Soybean	CO 1	0.683	0.417	0.310	0.470	1.663	1.263	1.177	1.368	1.417	1.193	1.080	1.230	1.023
	CO 2	0.760	0.367	0.323	0.483	1.657	1.290	1.063	1.337	1.353	1.163	0.883	1.133	0.984
	Mean	0.722	0.392	0.317	0.477	1.660	1.277	1.120	1.352	1.385	1.178	0.982	1.182	
CD (P=0.05)		V	T	S		VXT	TXS	VXS		VXTXS				
		0.102	0.126	0.126		0.178	0.218	0.178		0.308				
Green gram	CO 4	0.327	0.160	0.113	0.200	0.897	0.453	0.267	0.539	0.673	0.436	0.300	0.470	0.403
	VBN I	0.390	0.183	0.170	0.248	0.990	0.436	0.263	0.563	0.597	0.347	0.297	0.413	0.408
	Mean	0.358	0.172	0.142	0.224	0.943	0.445	0.265	0.551	0.635	0.392	0.298	0.442	
CD (P=0.05)		V	T	S		VXT	TXS	VXS		VXTXS				
		0.062	0.076	0.076		0.108	0.133	0.108		0.188				

Fig. 7. Effect of flood stress on Leghaemoglobin content (mg. g⁻¹ of dry nodule)



lesser content of leghaemoglobin followed by pod filling and flowering stages. Interaction effects were non-significant.

Soybean

As like that of cowpea, no significant difference between the varieties. Flooding reduces the content of leghaemoglobin at all the growth stages. The variety CO 2 (58%) had higher reduction of leghaemoglobin content as compared to CO 1 (55%) under 10 days of flooding at vegetative stage. Among the treatments, longer duration of flooding (10 days) reduces leghaemoglobin content in the per cent of 56 compared to shorter duration of flooding (5 days) had 45 per cent at vegetative stage. At growth stages, flowering recorded higher content of leghaemoglobin followed by pod filling and vegetative stage. Interaction effects were non significant.

Green gram

Same as that of cowpea and soybean, the green gram had no significant difference between the varieties for leghaemoglobin content. Flood stress decreases the content of leghaemoglobin at all the growth stages. Significant difference were observed between treatments and stages. The treatment flooding for 10 days recorded higher reduction of leghaemoglobin content compared to 5 days of flooding at flowering and vegetative followed by pod filling stages. At different growth stages, vegetative showed lesser content of leghaemoglobin was compared to flowering and pod filling stages. Interaction between the treatment and stage were significantly shown that flooding for 10 days reduces the content of leghaemoglobin was 71.9 per cent compared to flooding for 5 days (52.8%) of flowering stages.

4.2.2.4. Nitrogenase activity ($\mu\text{M C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{hr}^{-1}$) (Table 11; Fig. 8)

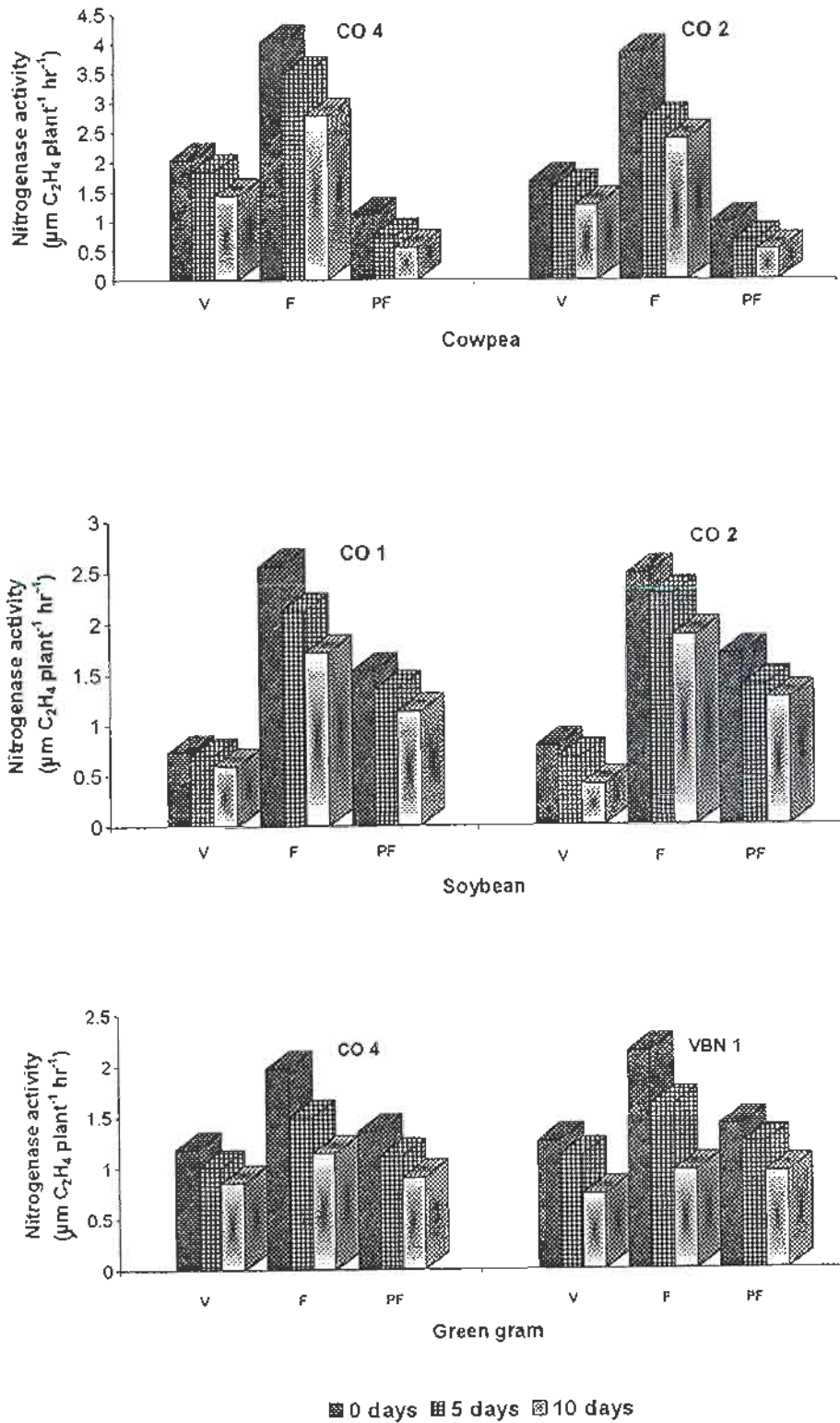
Cowpea

The acetylene reduction activity increased upto flowering thereafter it declined. There was significant difference between the varieties. The CO 4 (2.00) showed higher nitrogenase activity than CO 2 (1.74). Flooding significantly decreases the activity of nitrogenase in both the varieties. Among the treatments, the flooding for 10 days had more reduction of nitrogenase activity (35.5%) compared to 5 days

Table 11. Effect of flood stress on nitrogenase activity ($\mu\text{m C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	2.03	1.85	1.44	1.77	4.03	3.49	2.78	3.43	1.11	0.75	0.55	0.80	2.00
	CO 2	1.69	1.57	1.28	1.51	3.84	2.68	2.39	2.97	0.99	0.67	0.51	0.72	1.74
	Mean	1.86	1.71	1.36	1.64	3.94	3.09	2.57	3.20	1.05	0.71	0.53	0.76	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.114	0.140	0.140		0.195	0.242	0.097		0.343				
Soybean	CO 1	0.72	0.69	0.59	0.66	2.55	2.12	1.71	2.13	1.53	1.35	1.13	1.34	1.38
	CO 2	0.78	0.67	0.40	0.62	2.48	2.27	1.87	2.21	1.68	1.38	1.25	1.44	1.42
	Mean	0.75	0.68	0.50	0.64	2.52	2.20	1.79	2.17	1.61	1.37	1.19	1.39	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.109	0.134	0.134		0.183	0.216	0.183		0.311				
Green gram	CO 4	1.18	1.00	0.85	1.01	1.97	1.49	1.14	1.53	1.35	1.11	0.90	1.12	1.22
	VBN 1	1.25	1.11	0.73	1.03	2.13	1.61	0.96	1.57	1.41	1.23	0.94	1.19	1.26
	Mean	1.22	1.06	0.79	1.02	2.05	1.55	1.05	1.55	1.38	1.17	0.92	1.16	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.096	0.112	0.112		0.164	0.223	0.164		0.296				

Fig. 8. Effect of flood stress on Nitrogenase activity ($\mu\text{m C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$)



V - Vegetative; F - Flowering; PF - Pod Filling

flooding (19.3) over control. Regarding growth stages, flowering showed more activity of nitrogenase followed by pod filling and vegetative stage. Interaction between treatments and stages revealed that flooding for 10 days showed higher reduction in nitrogenase activity (34.8%) compared to 5 days flooding (21.6%) over control at flowering stage.

Soybean

Significant difference was found between the varieties, the mean value of nitrogenase activity was highest in CO 2 (1.42) and lowest in CO 1 (1.38). Flooding significantly decreases the activity of nitrogenase. Irrespective of the varieties, the flooding for 10 days recorded lower activity of nitrogenase (1.16) compared to 5 days flooding (1.42) and over control (1.63). Irrespective of the treatments, flowering showed higher activity of nitrogenase (2.17) followed by pod filling and vegetative stage. Interaction between treatments and stages indicated that flooding for 10 days recorded higher reduction in nitrogenase activity (24%) than 5 day flooding (12.7%) at flowering stage.

Green gram

There was no significant difference between the varieties. Flooding decreases the nitrogenase activity in both the varieties (CO 4 and VBN 1). Irrespective of the varieties, the flooding for 10 days recorded lower activity in nitrogenase (0.92) compared to 5 days flooding (1.26) over control (1.55). At different stages, the flowering recorded higher activity in nitrogenase (1.55) followed by pod filling (1.16) and vegetative (1.02) stage. Interaction between the varieties and treatments showed that VBN 1 had higher reduction in nitrogenase activity (16.9%) than CO 4 (36%) under 10 days flooding. Interaction between treatments and stages revealed that flooding for 10 days showed higher reduction in nitrogenase activity (48.8%) than 5 days flooding (24.4%) at flowering stage.

4.2.3. Growth attributes

4.2.3.1. Leaf area ($\text{cm}^2 \text{ plant}^{-1}$) (Table 12; Fig. 9)

Cowpea

Steady increase in leaf area was observed upto pod filling stage. Irrespective of the treatments the variety CO 2 recorded maximum leaf area compared to CO 4 at all stages. The maximum leaf reduction were recorded in CO 4 (24.8%) compared to CO 2 (10.87%) by 10 days of flooding at pod filling stage. Irrespective of the varieties the treatment flooding for 10 days had more leaf reduction (17.8%) compared to 5 days flooding (7.9%) at pod filling stage. Among the stages, the pod filling stages had higher leaf area followed by flowering and vegetative stages. Interaction effects were also found to be significant.

Soybean

Leaf area (130-141) low in the initial stages and increased rapidly (367-450) upto pod filling stage. The mean leaf area was observed more in CO 1 (343) compared to CO 2 (283). The influence of flooding on leaf area decreases in both the varieties (CO 1 and CO 2). The maximum leaf area was recorded at pod filling stage in CO 1 (450) compared to CO 2 (367). Treatment effects were non-significant. Among the treatments flooding for 10 days recorded reduction in leaf area was 8.9 per cent compared to 5 days flooding (5.9%). Irrespective of the stages the pod filling stage recorded more leaf area (408.5) compared to vegetative stage (135.1). Interaction effects between varieties and stages were showed that leaf area of CO 2 was significantly less (130) as compared to CO 1 (141) at vegetative stage.

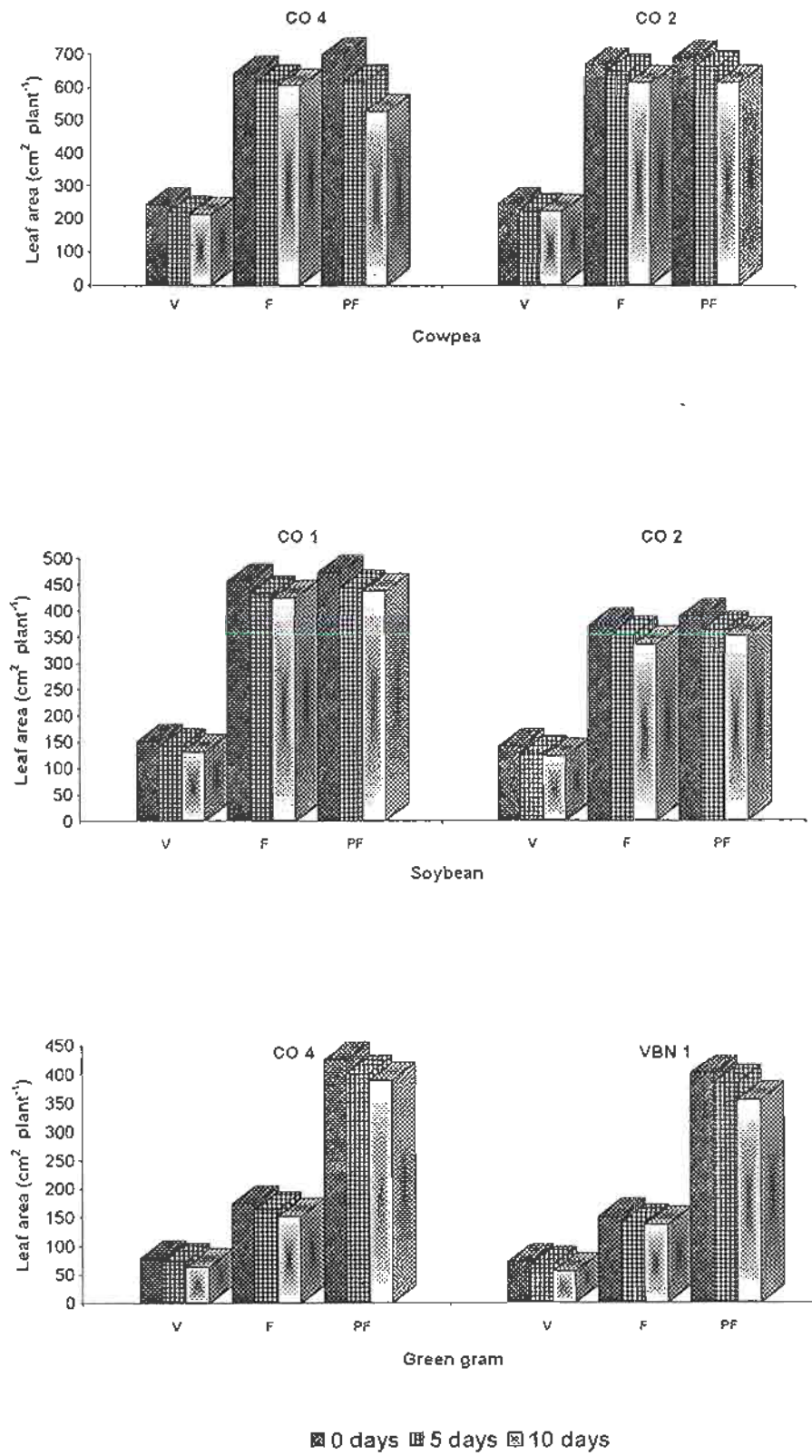
Green gram

The area of leaves increased from vegetative stage (68.0) reaching its peak at pod filling stage (392.4) and there after it declined. The mean leaf area varied from 206.6 (VBN 1) to 212.4 (CO 4) cm^2 per plant. There is no significant difference observed between stages of the crop. But leaf area was reduced significantly at all stages. Significant difference was observed between the treatments of the crop. Among treatments, flooding for 10 days recorded more reduction in leaf area (11.5%) compared to 5 days flooding (5.4%). Interaction effects were non-significant.

Table 12. Effect of flood stress on leaf area (cm² · plant⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	241.1	217.7	213.7	224.2	643.6	617.8	605.6	622.3	699.9	618.3	527.6	615.3	487.3
	CO 2	243.8	225.9	223.6	231.1	669.8	649.7	615.9	645.2	692.1	662.8	616.9	657.3	511.1
	Mean	242.5	221.8	218.9	227.6	656.7	633.8	610.8	633.8	696.0	640.5	572.3	636.3	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		22.897	28.044	28.044		39.660	48.573	39.660		68.693				
Soybean	CO 1	150.3	141.5	130.6	140.8	455.5	431.9	423.5	437.0	471.2	441.8	436.6	449.9	342.5
	CO 2	140.2	127.3	121.0	129.5	370.3	352.8	335.1	352.7	387.5	362.7	351.5	367.2	283.2
	Mean	145.3	134.4	125.8	135.1	412.9	392.4	379.3	394.9	429.4	402.3	394.0	408.5	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		22.845	27.974	27.979		39.569	48.462	39.569		68.536				
Green gram	CO 4	78.2	73.4	63.7	71.8	173.0	162.3	150.8	162.0	424.7	401.4	388.9	404.9	212.9
	VBN 1	71.8	66.7	54.8	64.2	150.7	139.7	136.4	142.3	400.2	384.1	354.9	379.7	206.6
	Mean	75.1	70.1	59.2	68.0	161.9	151.1	143.9	152.1	412.4	392.8	371.9	392.4	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		22.117	27.088	27.088		38.308	46.918	38.308		66.352				

Fig. 9. Effect of flood stress on Leaf area ($\text{cm}^2 \text{ plant}^{-1}$)



V - Vegetative; F - Flowering; PF - Pod Filling

4.2.3.1. Specific leaf weight (mg. cm²) (Table 13)

Cowpea

Specific leaf weight (SLW) was computed from the values of leaf dry weight and leaf area. SLW progressively increased from vegetative stage to pod filling stage. There was no significant difference between the varieties. The highest SLW recorded in CO 4 was 6.32 and 6.12 in CO 2. Irrespective of the treatment the maximum decrease because of SLW on 10 days of flooding at flowering stage in both the varieties. There is significant difference between the treatments at all stages. Among the treatments, flooding for 10 days (5.61) decreased the SLW compared to 5 days flooding (5.87) at flowering stage. Irrespective of the treatment the pod filling stage recorded more SLW followed by other stages. Interaction effects were nonsignificant.

Soybean

The mean SLW was significantly different from the varieties. Flooding treatment induces the SLW significantly in both varieties (CO 1 and CO 2). The variety CO 1 (5.33) recorded more SLW compared to CO 2 (4.60). Flooding for 10 days recorded more reduction of SLW in CO 2 (24.3%) compared to CO 1 (13%) at vegetative stage. Irrespective of the stages flooding for 10 days decreased the leaf area effectively compared to 5 days flooding. Irrespective of the treatment, the pod filling had more SLW compared to other growth stages. Interaction effects were non-significant.

Green gram

Significant difference existed in both varieties (CO 4 and VBN 1) for SLW. The variety VBN 1 (4.95) recorded more meaner SLW compared to CO 4 (4.04). Flooding treatment decreases SLW in both the varieties. Maximum specific leaf weight reduction in CO 4 (33%) compared to VBN 1 (27.5%) on 10 days flooding at vegetative stage. Irrespective of the varieties, the flooding for 10 days (24.8%) was recorded higher reduction of SLW compared to 5 days flooding at vegetative stage. Among the stages; the pod filling (12.8%) stage recorded more SLW followed by flowering the vegetative stage. Interaction effects were non-significant.

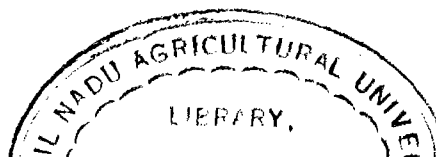


Table 13. Effect of flood stress on specific leaf weight (mg. cm⁻²) in pulses at different growth stages

Pulses	Vegetative stage				Flowering stage				Pod filling stage				Genotypes Mean	
	Control		Flooding for 10 days		Control		Flooding for 10 days		Control		Flooding for 10 days			Mean
	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean		
Cowpea	CO 4	6.57	6.25	5.92	6.25	6.89	6.15	5.65	6.21	7.06	6.52	5.88	6.49	6.32
	CO 2	6.47	6.00	5.58	6.02	6.80	5.60	5.57	5.99	6.84	6.45	5.86	6.38	6.13
	Mean	6.52	6.13	5.75	6.13	6.82	5.87	5.61	6.10	6.95	6.48	5.87	6.43	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	0.240	0.295	0.295		0.417	0.511	0.417		0.723					
Soybean	CO 1	5.47	5.02	4.76	5.08	5.72	5.51	5.13	5.45	5.85	5.53	5.01	5.46	5.33
	CO 2	4.73	4.09	3.58	4.14	4.89	4.51	4.56	4.66	5.44	5.00	4.63	5.02	4.60
	Mean	5.10	4.55	4.17	4.61	5.30	5.01	4.84	5.05	5.65	5.26	4.82	5.24	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	0.255	0.312	0.312		0.441	0.541	0.441		0.765					
Green gram	CO 4	4.55	3.99	3.09	3.86	4.29	3.52	3.58	3.80	5.30	4.13	3.96	4.46	4.04
	VBN 1	4.69	4.08	3.40	4.06	5.13	4.55	4.00	4.56	4.89	4.00	4.45	4.78	4.45
	Mean	4.62	4.03	3.24	4.00	4.71	4.03	3.79	4.18	5.10	4.07	4.20	4.62	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	0.331	0.405	0.405		0.573	0.702	0.573		0.993					

4.2.3.2. Specific leaf area ($\text{cm}^2 \text{mg}^{-1}$) (Table 14)

Cowpea

The specific leaf area (SLA) showed inverse relationship with specific leaf weight, with maximum (350) at vegetative stage and progressively decreasing upto pod filling stage (287). Significant differences were observed between the stages for SLA. The mean SLA of the varieties ranged from CO 2 (324.9) and to CO 4 (313.6) and difference were significant. Flooding treatment decreases SLA in both the varieties. More decrement of SLW observed in CO 2 compared to CO 4 at flowering stage by 10 days flooding. Among the treatments, the flooding for 10 days recorded lesser SLA compared to 5 days flooding at all growth stages. Irrespective of the treatment the pod filling stage recorded lesser SLA compared to other growth stages. Interaction effects were found to be significant.

Soybean

SLA showed maximum at vegetative stage and then it was declined gradually. The mean SLW was recorded highest in CO 1 (164.8) and lowest in CO 2 (160.4). SLA reduced due to flooding treatments in both the varieties. The maximum reduction of SLW observed in CO 1 variety (28.3%) compared to CO 2 (15.1%) at flowering stage due to 10 days flooding. Irrespective of the varieties, the flooding for 10 days (30.5%) decreases the SLW effectively compared to 5 days of flooding (7.8%) at flowering stage. Irrespective of treatment, the pod filling stages recorded lesser SLA compared to other growth stages. Interaction effects were found to be significant.

Green gram

Flooding treatment decreases the SLA. Significant difference was observed between the varieties for SLA. The variety VBN 1 had maximum decrement in SLA stage compared to CO 4 at vegetative stage by flooding for 10 days. Irrespective of the varieties, the flooding for 10 days (14.9%) recorded higher reduction of SLA compared the 5 days flooding (9.5%) at vegetative stage. Among the stages, the vegetative stage showed higher SLA followed by flowering and pod filling stage. Interaction between varieties and treatments showed that VBN 1 had higher reduction in SLA (13.5%) compared to CO 4 (10.1%) on 10 days flooding. Interaction between

treatments and stages revealed that flooding for 10 days recorded lower SLA (256.40) than 5 days flooding (201.20) at vegetative stage.

4.2.4. Total dry matter accumulation (TDMA) (g plant⁻¹) (Table 15; Fig. 10)

Cowpea

Dry matter is an important criterion as it determines the source-sink relationship. Varieties differed significantly for TDMA at all growth stages. The variety CO 2 (5.222) recorded higher TDMA than CO 4 (4.032) at all the growth stages except at vegetative stage. Flooding during all growth stages significantly reduced the TDMA. The difference in TDMA due to flooding was significant at all the growth stages. Dry matter reduction from 9.7 to 31.2 and 17.1 to 42.7 per cent due to 5 days and 10 days flooding respectively. The flooding of 10 days was more harmful. Among the stages, the vegetative stage recorded more reduction of TDMA at both the flooding treatments. Interaction effects due to varieties and stages were significantly showing higher TDMA in CO 2 (6.212) than CO 4 (3.640) at flowering stages.

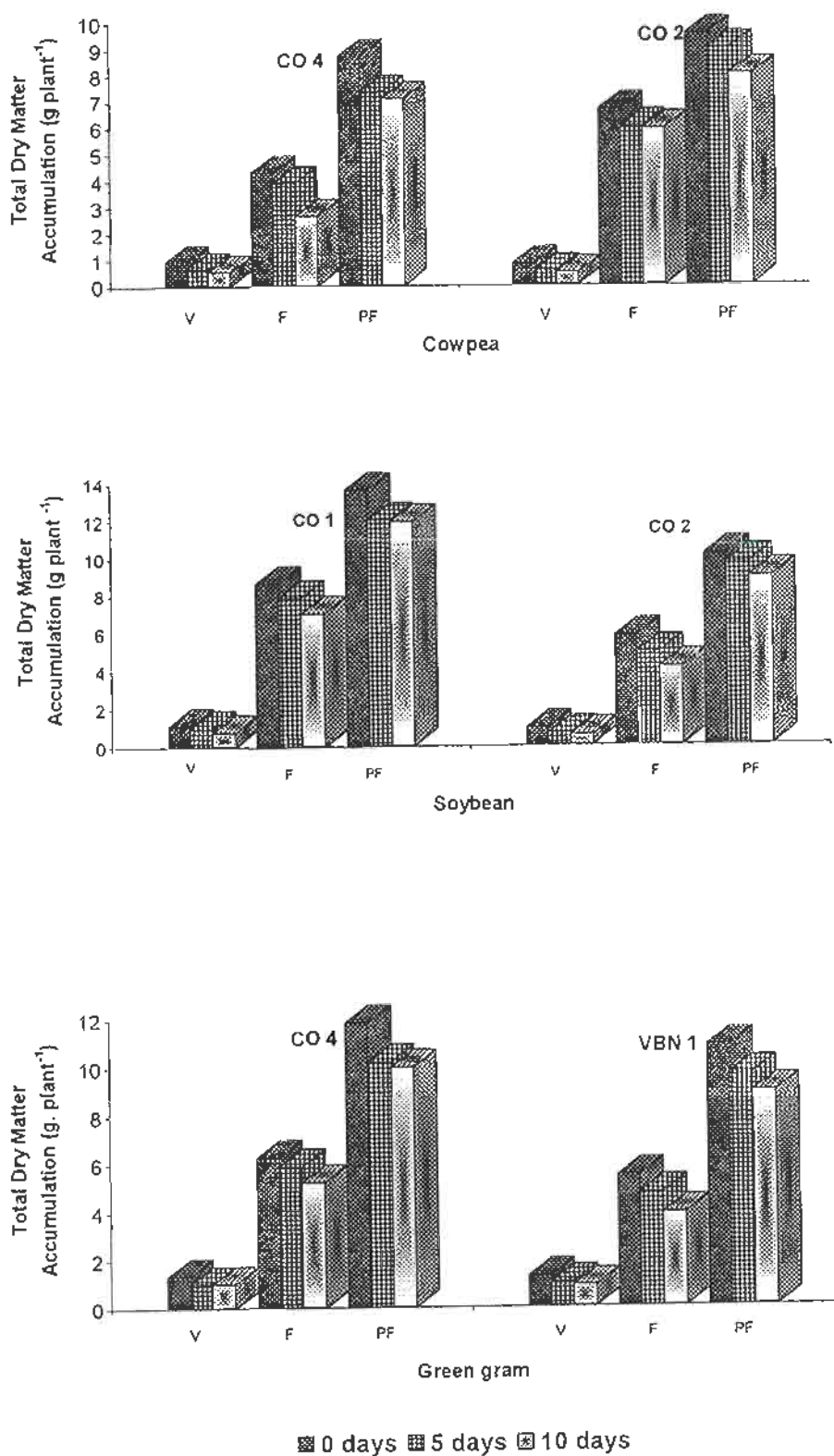
Soybean

Varieties different significantly for TDMA at all the growth stages. The variety CO 2 (7.109) recorded higher mean of TDMA than CO 1 (5.081). All flooding treatments significantly reduce TDMA in both the varieties. Irrespective of the varieties, the flooding for 10 days recorded lesser TDMA compared to 5 days flooding at all growth stages. Irrespective treatments, the pod filling stage had less affected in TDMA by flooding treatments compared to other growth stages. All interaction effects were found to be significant.

Green gram

Varieties differed significantly for TDMA at all growth stages. While comparing the varieties regarding TDMA the variety CO 4 recorded greater dry matter accumulation over the other variety VBN 1. The increase was 11.5 per cent. Irrespective of the varieties, the flooding treatment exhibited significant influence on TDMA. Plants flooded for 10 days was recorded less amount of TDMA in VBN 1

Fig. 10. Effect of flood stress on Total Dry Matter Accumulation (g plant^{-1})



V - Vegetative; F - Flowering; PF - Pod Filling

compared to CO 4. Among the treatments, flooding for 10 days (27.8%) recorded more reduction of TDMA compared to 5 days flooding at vegetative stage. Irrespective the treatment, the TDMA more at pod filling stages (10.242) followed by flowering (5.243) and vegetative (1.091) stages. Interaction effects were found to be significant.

4.2.5. Physiological changes - Leaf gas exchange parameters

4.2.5.1. Photosynthetic rate (μ mole $\text{CO}_2 \text{ m}^{-1} \text{ s}^{-2}$) (Table 16; Fig. 11)

Cowpea

There is no significant variation in photosynthetic rate (Pn) of cowpea varieties (CO 2 and CO 4). Flooding treatment significantly reduces Pn at all stages. Irrespective of the genotypes, the flooding for 10 days significantly decreases Pn compared to 5 days flooding in both varieties at all growth stage. The reduction of Pn was highest in 10 days flooding (47.3%) compared to flooding for 5 days (33.7%) at flowering stage. Different growth stages concerned, the flooding at flowering stage showed more decrement in Pn. Lowest Pn rate was recorded at pod filling stage. Interaction effects were non-significant.

Soybean

No significant difference between the varieties for photosynthetic rate. Flood stress decreases Pn rate at all growth stages. The difference in Pn between the treatments was found to be significant. Flooding for 10 days (14.6) was shown lesser Pn rate compared to 5 days flooding (17.7) over control (26.7) at flowering stage. Irrespective of the treatment, the flowering stage recorded higher Pn rates followed by vegetative and pod filling stages. Interaction between treatment and stages showed that flooding for 10 days recorded higher reduction in Pn was 45.3% compared to 5 days flooding (33.6%) at vegetative stage.

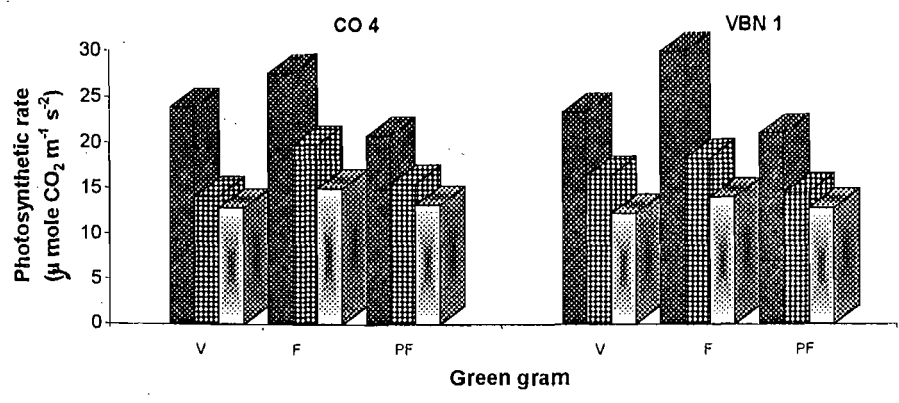
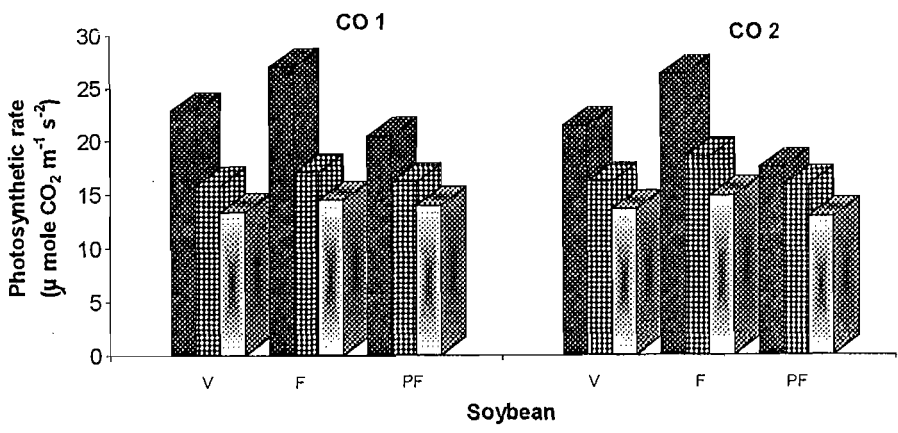
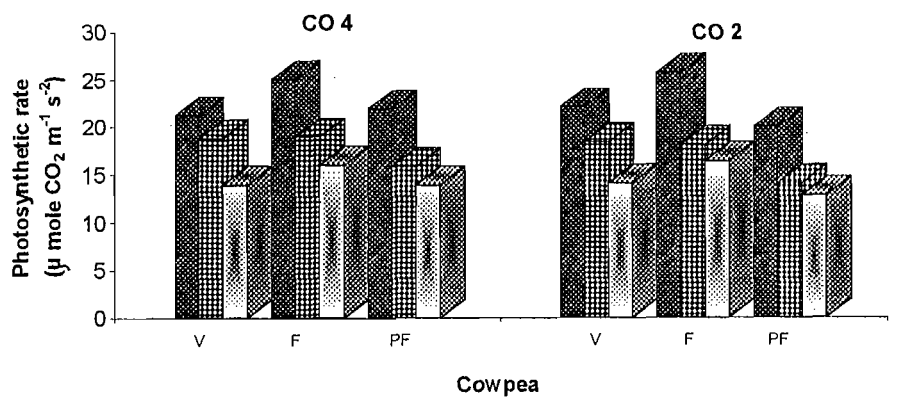
Green gram

There is no significant difference existed in both the varieties (CO 4 and VBN 1) for Pn rate. The mean Pn rate of CO 4 was at par with the VBN 1. The variety VBN 1 had more reduction of Pn rate compared to CO 4 on 10 days flooding at all

Table 16. Effect of flood stress on photosynthetic rate (μ mole $\text{CO}_2 \text{ m}^{-1} \text{ s}^{-2}$) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean					
	Control	Flooding for 5 days	Flooding for 10 days	Control	Flooding for 5 days	Flooding for 10 days	Control	Flooding for 5 days	Flooding for 10 days		Mean				
Cowpea	CO 4	21.2	18.8	13.9	18.0	25.1	19.0	16.0	20.0	20.0	21.9	15.8	13.9	17.2	18.4
	CO 2	22.1	18.4	14.1	18.2	25.8	18.1	16.4	20.1	20.1	20.0	14.1	12.8	15.6	18.0
	Mean	21.7	18.6	14.0	18.1	25.4	18.6	16.2	20.1	20.1	20.9	15.0	13.4	16.4	
CD (P=0.05)	V	1.096	1.342	S	1.342	VXT	TXS	VXS	VXTXS	3.286					
	T	1.342	1.342	S	1.342	1.898	2.325	1.898							
	S	1.342	1.342	1.342	1.342	1.898	1.898	1.898							
Soybean	CO 1	22.8	15.8	13.3	17.3	27.0	17.0	14.4	19.5	19.5	20.4	16.1	13.9	16.8	17.9
	CO 2	21.3	16.1	13.5	17.0	26.3	18.4	14.8	19.9	19.9	17.4	15.8	12.8	15.3	17.4
	Mean	22.1	16.0	13.4	17.1	26.7	17.7	14.6	19.7	19.7	18.9	16.0	13.4	16.1	
CD (P=0.05)	V	1.089	1.334	S	1.334	VXT	TXS	VXS	VXTXS	3.267					
	T	1.334	1.334	S	1.334	1.886	2.310	1.886							
	S	1.334	1.334	1.334	1.334	1.886	1.886	1.886							
Green gram	CO 4	23.8	14.1	12.8	16.9	27.4	19.6	14.9	20.6	20.6	20.6	15.4	13.1	16.4	18.0
	VBN 1	23.1	16.3	12.1	17.2	29.6	18.1	13.9	20.5	20.5	20.8	14.4	12.7	16.0	17.9
	Mean	23.5	15.2	12.3	17.0	28.5	18.9	14.4	20.6	20.6	20.7	14.9	12.9	16.2	
CD (P=0.05)	V	1.094	1.340	S	1.340	VXT	TXS	VXS	VXTXS	3.283					
	T	1.340	1.340	S	1.340	1.895	2.321	1.895							
	S	1.340	1.340	1.340	1.340	1.895	1.895	1.895							

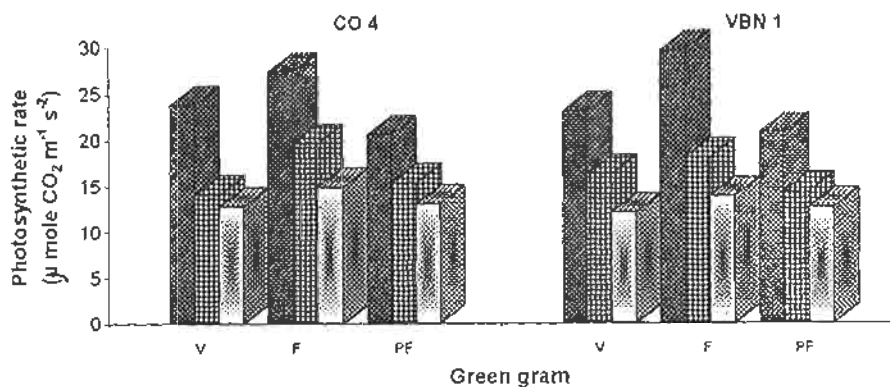
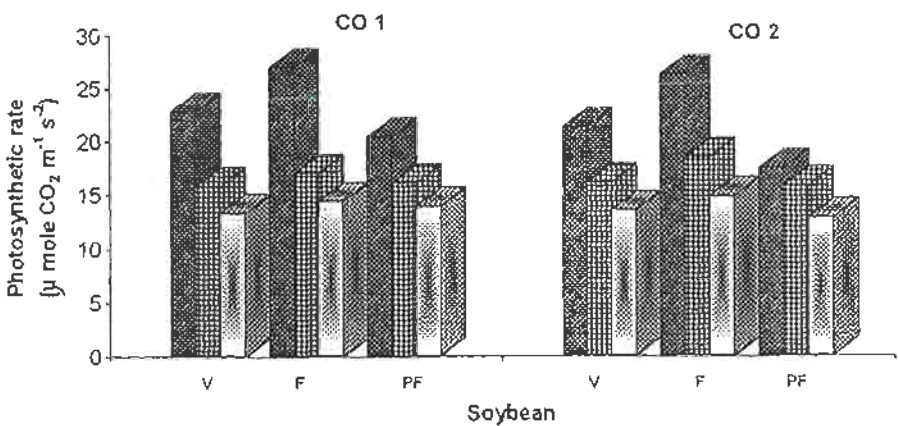
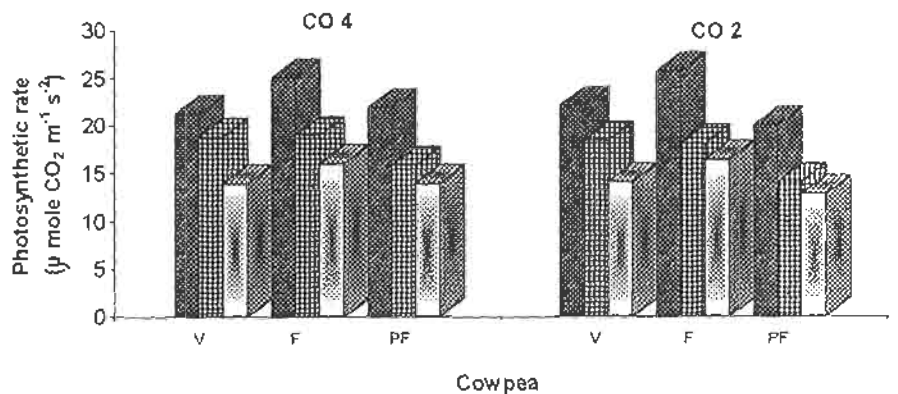
Fig. 11. Effect of flood stress on Photosynthetic rate (μ mole $\text{CO}_2 \text{ m}^{-1} \text{ s}^{-2}$)



■ 0 days ■ 5 days ■ 10 days

V - Vegetative; F - Flowering; PF - Pod Filling

Fig. 11. Effect of flood stress on Photosynthetic rate (μ mole $\text{CO}_2 \text{ m}^{-1} \text{ s}^{-2}$)



■ 0 days ■ 5 days □ 10 days

V - Vegetative; F - Flowering; PF - Pod Filling

growth stages. Irrespective of the varieties, the treatment flooding for 10 days were recorded significantly lesser Pn rate compared to 5 days flooding at all growth stages. Among the growth stages, the flowering stages were more susceptible for both flooding treatments compared to vegetative and pod filling stages. Interaction between treatments and stages revealed that the flooding for 10 days showed lower Pn (14.4) compared to 5 days flooding (18.9) over control (28.5) at flowering stages.

4.2.5.2. Transpiration rate (E) (μ moles H₂O m⁻² s⁻¹) (Table 17)

Cowpea

Mean transpiration rate increased steadily upto flowering stage and there after it will declined. The varietal difference for transpiration rate was non-significant. The mean transpiration rate of CO 2 was at par with CO 2 at all growth stages. Under flooding treatments the transpiration rate decreased significantly. Irrespective of the varieties, the treatments showed significant difference at all the stages. Among the treatments, the flooding for 10 days recorded lower transpiration rate (3.5) compared to 5 days flooding (4.6) over control (6.0) at pod filling stage. Irrespective of the treatments, the flowering stage recorded more transpiration rate (9.4) followed by vegetative (7.8) and pod filling (4.7) stage.

Soybean

No significant difference between the varieties for transpiration rate. The variety CO 2 recorded more mean transpiration rate (7.1) compared to CO 1 (6.7). Flooding treatment significantly decreased the transpiration rate in both the varieties (CO 1 and CO 2). At all growth stages, the flooding for 10 days recorded high reduction in transpiration rate ranges from 35.9 to 42.6 per cent compared to 5 days of flooding it ranges from 17.1 to 25.8 per cent. Irrespective of treatment, the pod filling stages showed lesser transpiration rate followed by vegetative and flowering stages. Interactions were non significant.

Green gram

There was significant difference existed between the varieties for transpiration rate. The mean transpiration rate of VBN 1 (8.1) showed higher rate of transpiration

Table 17. Effect of flood stress on transpiration rate (μ mole H_2O $m^{-1} s^{-2}$) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	9.8	7.2	6.4	7.8	11.5	9.3	8.3	9.7	6.4	4.5	3.8	5.0	7.4
	CO 2	9.6	7.5	6.1	7.7	10.9	8.8	8.1	9.3	5.5	4.6	3.1	4.4	7.1
	Mean	9.7	7.3	6.3	7.8	11.2	9.0	8.2	9.5	6.0	4.6	3.5	4.7	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.729	0.893	0.893		1.263	1.547	1.263		2.188				
Soybean	CO 1	8.4	6.6	5.7	6.9	9.8	7.3	6.5	7.9	6.6	5.4	4.0	5.3	6.7
	CO 2	9.5	6.7	4.5	6.9	10.8	7.5	6.7	8.4	7.4	6.2	4.3	5.9	7.1
	Mean	8.9	6.6	5.1	6.9	10.3	7.4	6.6	8.1	7.0	5.8	4.2	5.6	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.734	0.899	0.899		1.271	1.577	1.271		2.203				
Green gram	CO 4	5.3	4.0	3.5	4.2	6.4	3.5	3.1	4.3	4.0	2.8	2.2	3.0	3.9
	VBN 1	9.7	7.6	7.1	8.1	11.0	7.9	7.8	8.9	8.4	7.2	6.4	7.3	8.1
	Mean	7.5	5.8	5.3	6.2	8.7	5.7	5.4	6.6	6.2	5.0	4.3	5.1	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.715	0.876	0.876		1.239	1.518	1.239		2.460				

compared to CO 4 (3.9). Flooding treatment significantly decreases the transpiration rate in both varieties. Among the varieties, CO 4 (51.6%) showed more reduction in rate of transpiration compared to VBN 1 (29%) on 10 days flooding at flowering stages. The flooding for 10 days (5.4) significantly reduces the rate of transpiration compared to 5 days flooding (5.7) over to control (8.7) at flowering stage. The different growth stages, the pod filling stage (5.1) recorded lesser rate of transpiration followed by vegetative (6.2) and flowering stage (6.6). Interaction effects were found to be non significant.

4.2.5.3. Stomatal resistance (r_s) ($s\ m^{-1}$) (Table 18)

Cowpea

The varieties varied significantly in their stomatal resistance (r_s). The mean r_s recorded highest (9.3) in CO 2 against the lowest (8.7) in CO 4. Flooding significantly increased the r_s value compared with control. Under flooding treatments, the variety CO 4 showed higher per cent of r_s (61.1%) compared to CO 2 (53.3%) on 10 days of flooding at vegetative stage. Irrespective of the varieties, the flooding for 10 days (56.1) recorded highest per cent of r_s compared to 5 days flooding (26.3) at vegetative stages. Among the stages the higher mean r_s recorded at pod filling stages (10.4) were compared to flowering (9.3) and pod filling stages (7.3). Interaction effects were non-significant.

Soybean

Varieties differed significantly in their r_s . The mean r_s of CO 1 were on par with CO 2. Flooding stress significantly increases r_s in both varieties. Among the varieties, CO 2 (9.3) had slight increase of r_s compared to CO 1 (10.1) on 10 days flooding at vegetative stage. The flooding treatments especially longer duration of flooding (10 days) significantly increase as compared to 5 days of flooding at vegetative stages. Stomatal resistance decreased with the phenology of the crop was significant. At different growth stage, the pod filling stage recorded lower r_s value followed by vegetative and pods filling stage. Interaction between treatments and stages, variety and stage were significant.

Green gram

Diffusive resistance shows the capacity of stomata to open or to close. Stomatal resistance increased when the plants are exposed to flooding. The mean value of r_s was higher in CO 4 (8.9) compared to VBN 1 (5.0). Under flooded conditions especially 10 days flooding increases r_s in VBN 1 compared to CO 4 variety at all growth stages. Flooding for 10 days significantly increases r_s compared to 5 days flooding over control at flowering stage. The flooding for 10 days was more harmful to r_s . Among the growth stages, the vegetative stage recorded lower r_s value compared to flowering and pod filling stage. Interaction between variety and treatment was significantly showing by higher r_s in CO 4 than VBN 1 due to flooding for 10 days. Where as interaction between variety and stages revealed most severe effect of 10 days flooding was 55.4% over control at flowering stage.

4.2.5.4. Water Potential (-MPa) (Table 19)

Cowpea

Leaf water potential tends to decrease over the growth stage. The difference between the varieties for water potential was non-significant. Flooding treatments decreased the leaf water potential (LWP) at all growth stages. Among the flooding treatment, flooding for 10 days recorded higher LWP (-2.07) decrement as compared to 5 days flooding (-0.98) at flowering stage. Non significant CO 2 recorded lesser leaf water potential as compared to CO 1 at all growth stages. At different growth stages, vegetative stage recorded higher LWP as followed by pod filling and flowering stages. Interaction effects were non-significant.

Soybean

The difference between the varieties for LWP was non-significant. Flooding stress decrease LWP in both varieties (CO 1 and CO 2). Flooding for 10 days recorded higher reduction of LWP as compared to 5 days flooding as over to control at vegetative stage next to flowering stage. Among the growth stages, the flowering and pod filling stages were on par with each other and significantly recorded more LWP than vegetative stage. Non significant interaction effects were found.

Green gram

Non significant difference between the varieties and stages for LWP. Flooding reduces the LWP in both varieties (CO 4 and VBN 1) at all the growth stages. Among different duration of flooding the 10 days flooding decreases LWP (-1.64) as compared to 5 days flooding (-0.89) over to control (-0.51) at vegetative stage. At different growth stages, the vegetative, flowering and pod filling stages were at par with each other in LWP. Interaction effects were found to be non significant.

4.2.6. Chlorophyll content

4.2.6.1. Chlorophyll 'a' content (mg. g⁻¹) (Table 20)

Cowpea

The maximum Chl a content was recorded at flowering stage (1.026). Significant difference was existed between the varieties. The mean chl a content was recorded higher in CO 2 (0.911) compared to CO 4 (0.823). The variety CO 2 had lower content of Chl a compared to CO 4 on 10 days of flooding at flowering stage. The flooding for 10 days (34.5%) recorded significantly higher reduction of chlorophyll a content compared to 5 days flooding (8.4%) at flowering stage. In the growth stages, the flowering recorded higher chl contents followed by vegetative and pod filling stages in both varieties. Interaction effects between treatments and stages showed that flooding for 10 days recorded higher reduction in chl a content was 34.6% compared to 5 days flooding at flowering stages.

Soybean

Significant difference was observed between the varieties for chl a content. The mean values for chl a content was recorded higher in CO 2 (1.074) compared to CO 1 (0.960). Flooding decreases chl a content in both the varieties. Flooding for longer duration (10 days) decreased the Chl a content in CO 2 (53.1%) compared to CO 1 (45.3) over control at flowering stage. Among the flooding treatment, the 10 days of flooding (50%) showed higher reduction of chlorophyll a content compared to flooding for 5 days (26%) to over control at flowering stage. In the growth stages, flowering was most sensitive to flooding treatments and also recorded

higher chlorophyll a content followed by vegetative and pod filling stages. Interaction effects between variety and treatment was significant showing reduction in Chl by 2.41% in CO 1 than CO 2 due to flooding for 10 days whereas in interaction between treatment and stage was also significant.

Green gram

There is no significant difference between varieties for Chl a content. Flooding decreases Chl a content in both varieties (CO 4 and VBN 1). Flooding for longer duration (10 days) decreases Chl a content in VBN 1 (50%) compared to 20.2% in CO 4 at flowering stage. Among the flooding treatments, 10 days flooding had more reduction of Chl a content (39.7%) compared to flooding for 5 days (10.3%) at pod filling stage. Irrespective of the treatments, the flowering stage had recorded highest value of Chl a content followed by pod filling and vegetative stage. Interaction between variety and treatment were found to be significant.

4.2.6.2. Chlorophyll 'b' content (mg. g⁻¹) (Table 21)

Cowpea

Maximum Chl 'a' content was recorded at flowering stage (0.792). Significant difference was recorded between the varieties. The mean Chl b content was showed higher in CO 4 compared to CO 2 at all the growth stages. Flooding treatment reduces the Chl b content in both varieties. Higher reduction of Chl b contents recorded significantly in CO 2 (24.2%) compared to CO 4 (14.1%) on longer duration of flooding at flowering stage. Longer duration of flooding (10 days) recorded less Chl b content compared to 5 days of flooding at vegetative stages. Among the stages, flowering stage recorded higher content of Chl b (0.792) followed by vegetative (0.710) and pod filling (0.695) stages, respectively. Interaction effects between variety and treatment found to be significant.

Soybean

Significant difference was recorded between the varieties. The variety CO 2 (0.749) showed higher mean leaf Chl b content compared to CO 1 (0.698). The flooding stress decreases Chl b content in both varieties. Flooding for longer duration

(10 days) decreases Chl b content highest in CO 2 and lowest in CO 1 compared to shorter duration of flooding (5 days) at all growth stages. The different duration of flooding, flooding for 10 days (19.5%) had more reduction of chlorophyll b content compared to 5 days flooding (6.6%) at flowering stage. In the growth stages, flowering was very sensitive to different flooding duration followed by vegetative and pod filling stage. Interaction between variety and treatment, variety and stage, variety and treatment and stage were in significant.

Green gram

The difference in Chl b content was significant among the varieties, treatment and stages. The variety CO 4 (0.523) recorded higher content of Chl b than the VBN I (0.471). Flooding treatments reduces Chl b content in both varieties. Among the flooding duration, higher duration of flooding (10 days) recorded higher reduction of Chl b content (32.7%) than 5 days of flooding (21.5) at flowering stage. At different growth stages, flowering stage were recorded higher Chl b content than pod filling and vegetative stage. Interaction between treatment and stage, variety and stage were significant.

4.2.6.3. Total chlorophyl (mg g^{-1}) (Table 22)

Cowpea

Significant difference was exists between the varieties for total chlorophyll content. The mean values for total chl content varied from 1.227 (CO 4) to 1.567 (CO 2). Flooding treatment decreased the total Chl content in both varieties. The decrease in total Chl content was maximum at vegetative stage as compared to flowering and pod filling stage. Longer period of flooding (10 days) at vegetative stage had detrimental effect on total Chl content shown in maximum reduction of 46.1 per cent. The variety CO 2 recorded significantly lower total Chl content in 10 days flooding as compared to CO 4 at vegetative stage. Among the growth stages, the flowering stage recorded higher total Chl content than the other growth stages. Interaction between treatment and stage were significant.

Soybean

Total Chl content steadily increased from vegetative stage and thereafter it declined at pod filling stage. The varietal difference for total Chl contents was non-significant. Flooding treatment decreases total Chl content in both varieties (CO 1 and CO 2). The variety CO 2 recorded lower content by total Chl in longer duration of flooding as compared to CO 1 at vegetative stage. Longer duration of flooding (10 days) recorded least content of total Chl than 5 days of flooding at vegetative stage. Regarding growth stages, the vegetative stage were sensitive to both flooding treatments followed by flowering and pod filling stages. Interaction effects were significant.

Green gram

Genotypic difference for total Chl contents was non-significant. Flooding treatment decreases total Chl content in both varieties (CO 1 and VBN 1). Under different duration of flooding the 10 days of flooding (33.3%) showed the more reduction in content of total chlorophyll as compared to flooding for 5 days (19.1%) at vegetative stage. Among different growth stages, the pods filling stage recorded lesser content of total Chl as compared to vegetative and pod filling stages. Interaction effects were non-significant.

4.2.7. Chlorophyll fluorescence

4.2.7.1. Variable fluorescence (fv) (Table 23)

Cowpea

Variable chlorophyll fluorescence (fv) increases upto flowering stage and thereafter it declined. Significant difference between the varieties for fv. The mean value of fv was recorded highest in CO 4 (1562.2) and lowest in CO 2 (1525.8). The variety CO 4 recorded maximum fv during flowering stage compared to CO 2. Flooding decreases fv value at all growth stages. Maximum reduction of fv was noticed in CO 4 (37.3%) as compared to CO 2 (13.1%) on 10 days flooding at vegetative stage. Among the treatments, the highest decrease of fv (34.1) was found in plants grown under 10 days of flooding at flowering stage. Regarding growth stages,

the mean of fv noticed less in pod filling stages followed by pod filling and vegetative stage. Interaction between variety and treatment, treatment and stage and variety, treatment and stage were significant.

Soybean

There is no significant difference between the varieties for fv. Flooding stress decreases fv value in both varieties (CO 1 and CO 2). Lower values of fv in both varieties were at par with each other during 10 days flooding at flowering stages. The treatment flooding for 10 days (1415) was recorded lowest value of fv as compared to 5 days flooding (1665) over control (2135.3) at flowering stage. Among the growth stages, flowering stage recorded highest value of fv (1738.4) followed by vegetative and pod filling (1274.2) stages. Interaction (1374.1) between treatment and stage revealed that flooding for 10 days recorded lower value of fv (1418) compared to 5 days flooding (1618.5) over control (2154.3) at flowering stage.

Green gram

Significant difference between the varieties for fv. Flooding stress decreases the fv value in both the varieties (CO 4 and VBN 1). The variety CO 4 (1511) exhibits a high mean value of fv as compared to VBN (1449). The maximum decrements of fv in VBN 1 with 10 days flooding as compared with CO 4 at vegetative and pod filling stages. However at pod filling stage the variety CO 4 had reverse in fv value. Flooding for 10 days recorded maximum reduction in fv as compared to 5 days flooding at pod filling (32.6 and 18.01 %) and flowering stages (31.6% and 22.2%). At different growth stages, flowering recorded highest value of fv as followed by vegetative and pod filling stage. All interaction effects were significant except interaction between variety and treatment.

4.2.7.2. Chlorophyll fluorescence -maximum fluorescence (fm) (Table 24)

Cowpea

Maximum fluorescence increases upto flowering stage and later it declined slightly (fm). No significant difference exists between the varieties for fm. All flooding duration decreases fm value considerably in both varieties. Among the

Table 24. Effect of flood stress on chlorophyll fluorescence-maximum fluorescence (fm) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	2264.7	2169.3	2030.0	2154.7	2466.7	2134.3	1965.0	2188.7	2144.7	1962.3	1758.0	1955.0	2099.4
	CO 2	2360.0	2014.0	2179.0	2184.4	2614.7	2151.0	1927.0	2230.9	2159.7	1924.3	1727.0	1937.0	2117.4
	Mean	2312.3	2091.8	2104.0	2169.6	2540.7	2142.7	1946.0	2209.8	2152.2	1943.3	1752.5	1946.0	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		49.603	60.751	60.751		85.915	105.225	25.915		148.810				
Soybean	CO 1	2287.7	2099.3	1979.0	2122.0	2477.6	2138.7	1977.0	2197.8	1924.7	1974.3	1665.0	1854.7	2058.1
	CO 2	2171.7	2019.7	1930.0	2040.4	2374.7	2054.3	1899.0	2109.3	1976.7	1896.3	1697.0	1856.7	2002.1
	Mean	2229.7	2059.5	1954.0	2081.2	2426.2	2096.5	1938.0	2153.6	1950.7	1935.3	1681.0	1855.7	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		49.770	60.955	60.955		86.204	105.578	86.204		149.310				
Green gram	CO 4	2390.7	2044.3	1949.0	2128.0	2640.7	2153.3	1937.0	2243.7	2154.7	1934.3	1679.0	1922.7	2098.1
	VBN I	2368.7	2017.3	1910.0	2098.7	2564.7	2153.3	1985.0	2234.3	2099.7	1982.3	1610.0	1897.3	2076.8
	Mean	2379.7	2030.8	1929.0	2113.3	2602.7	2153.3	1961.0	2239.0	2127.2	1958.3	1644.5	1910.0	
CD		V	T	S		VXT	TX S	VXS		VXTXS				
		49.277	60.352	60.352		85.351	104.533	85.351		147.833				

varieties, the CO 2 (26.3%) recorded lesser fm value as compared to CO 1 (20.3%) on 10 days of flooding at flowering stage. The flooding treatments like the 10 days of flooding (1946) recorded lowest value of fm as compared to 5 days flooding (2143) over control (2541) at flowering stage. Regarding growth stages, pod filling recorded lowest value of fm as compared to vegetative and flowering stage. Interaction between treatment and stage were significantly showed that most severe effect of 10 days flooding at flowering stage over control.

Soybean

Significant difference between the varieties for fm was noticed. The variety CO 1 (2058) recorded highest mean value of fm as compared to CO 2 (2002). Highest reduction was found in both varieties (20% and 20.2%) under 10 days flooding at flowering stage. Flooding for 10 days (1938) were recorded lowest value of fm as compared to 5 days over control (2426) at flowering stage. The different growth stages the pod filling recorded lesser value of fm as followed by vegetative and flowering stages. The magnitude of the effects of treatment was found less at later stages. Interaction between treatment and stage were significant.

Green gram

There is no significant difference between the varieties for fm value. Flooding duration affects the fm value in both varieties (CO 4 and VBN 1). Among the varieties, the CO 4 (26.6%) recorded higher reduction of fm value as compared to VBN 1 (22.6%) under 10 days of flooding at flowering stage. Different flooding treatments, flooding for 10 days recorded higher reduction of fm value (24.6%) as compared to 5 days flooding (17.2%) over control at flowering stage. Among the stages, pod filling recorded lowest value of fm as followed by vegetative and flowering stages. Interaction between treatment and stage were indicated that 10 days of flooding significantly reduces fm value by 24.7% as compared to 5 days flooding (17.3%) over control at flowering stage.

4.2.7.3. Chlorophyll fluorescence ratio (fv/fm) (Table 25; Fig 12)

Cowpea

The ratio of fv/fm value increases upto flowering and thereafter it declined. Significant difference between the varieties for fv/fm was significant. The variety CO 4 recorded highest mean value of fv/fm (0.737) as compared to CO 2 (0.715). The ratio of fv/fm was significantly decreased with the flooding treatments at all the growth stages. The variety CO 4 was with low ratio of fv/fm (30.1) over the variety CO 2 (6.0%) under 10 days of flooding at vegetative stage. Among the different duration of flooding, flooding for 10 days decreases the ratio of fv/fm as compared to 5 days flooding at all the growth stages. Flooding for 5 days had less harmful effects to ratio of fv/fm. At different growth stages, vegetative stage very sensitive to flooding followed by pod filling and flowering stages. However, the mean value of fv/fm was lowest in pod filling stage followed by vegetative and flowering stage. All interaction effects were significant.

Soybean

Varieties difference for fv/fm ratio was significant. The mean value of fv/fm was highest in CO 2 (0.725) as compared to CO 1 (0.705). The ratio of fv/fm was significantly decreased with different flooding treatments at all growth stages. The variety CO 1 had higher reduction of fv/fm ratio as compared to CO 2 on 10 days flooding at both flowering and pod filling stages. Among the treatment, flooding for 5 days recorded less reduction of fv/fm ratio (9.6%) as compared to 10 days flooding (16.9%) at flowering stage. At different growth stages, the vegetative stage recorded lower ratio of fv/fm, as followed by pod filling and flowering. Interaction effects were found to be significant.

Green gram

The difference between the varieties for fv/fm ratio was significant. The mean value of fv/fm ratio was highest in CO 4 (0.703) as compared to VBN 1 (0.691). The variety CO 4 was recorded lowest value in 10 days flooding as compared to VBN 1 at all growth stages except flowering stage. Among the flooding for different duration, higher duration of flooding (10 days) had detrimental effect on fv/fm ratio on

varieties, the CO 2 (26.3%) recorded lesser fm value as compared to CO 1 (20.3%) on 10 days of flooding at flowering stage. The flooding treatments like the 10 days of flooding (1946) recorded lowest value of fm as compared to 5 days flooding (2143) over control (2541) at flowering stage. Regarding growth stages, pod filling recorded lowest value of fm as compared to vegetative and flowering stage. Interaction between treatment and stage were significantly showed that most severe effect of 10 days flooding at flowering stage over control.

Soybean

Significant difference between the varieties for fm was noticed. The variety CO 1 (2058) recorded highest mean value of fm as compared to CO 2 (2002). Highest reduction was found in both varieties (20% and 20.2%) under 10 days flooding at flowering stage. Flooding for 10 days (1938) were recorded lowest value of fm as compared to 5 days over control (2426) at flowering stage. The different growth stages the pod filling recorded lesser value of fm as followed by vegetative and flowering stages. The magnitude of the effects of treatment was found less at later stages. Interaction between treatment and stage were significant.

Green gram

There is no significant difference between the varieties for fm value. Flooding duration affects the fm value in both varieties (CO 4 and VBN 1). Among the varieties, the CO 4 (26.6%) recorded higher reduction of fm value as compared to VBN 1 (22.6%) under 10 days of flooding at flowering stage. Different flooding treatments, flooding for 10 days recorded higher reduction of fm value (24.6%) as compared to 5 days flooding (17.2%) over control at flowering stage. Among the stages, pod filling recorded lowest value of fm as followed by vegetative and flowering stages. Interaction between treatment and stage were indicated that 10 days of flooding significantly reduces fm value by 24.7% as compared to 5 days flooding (17.3%) over control at flowering stage.

4.2.7.3. Chlorophyll fluorescence ratio (fv/fm) (Table 25; Fig 12)

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Soybean

Varieties difference for fv/fm ratio was significant. The mean value of fv/fm was highest in CO 2 (0.725) as compared to CO 1 (0.705). The ratio of fv/fm was significantly decreased with different flooding treatments at all growth stages. The variety CO 1 had higher reduction of fv/fm ratio as compared to CO 2 on 10 days flooding at both flowering and pod filling stages. Among the treatment, flooding for 5 days recorded less reduction of fv/fm ratio (9.6%) as compared to 10 days flooding (16.9%) at flowering stage. At different growth stages, the vegetative stage recorded lower ratio of fv/fm, as followed by pod filling and flowering. Interaction effects were found to be significant.

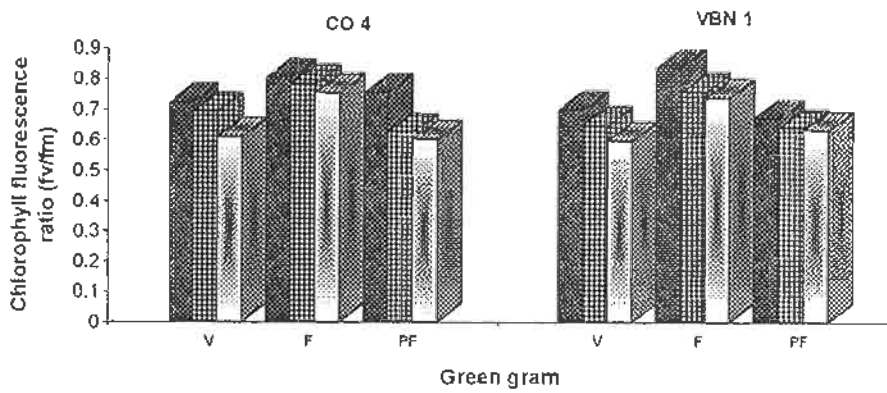
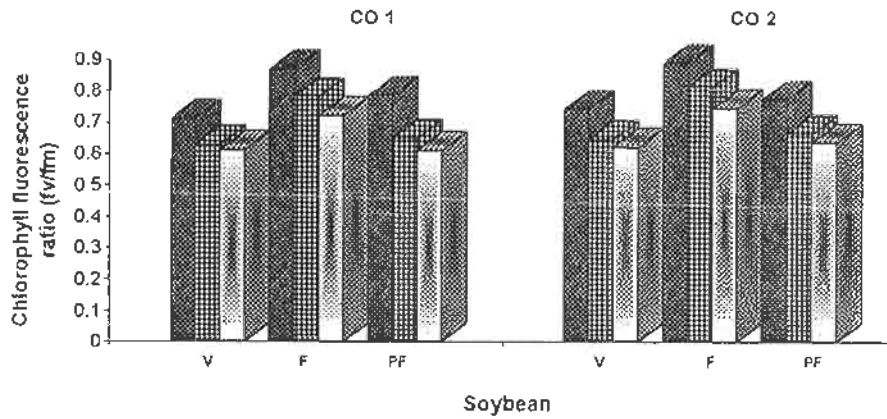
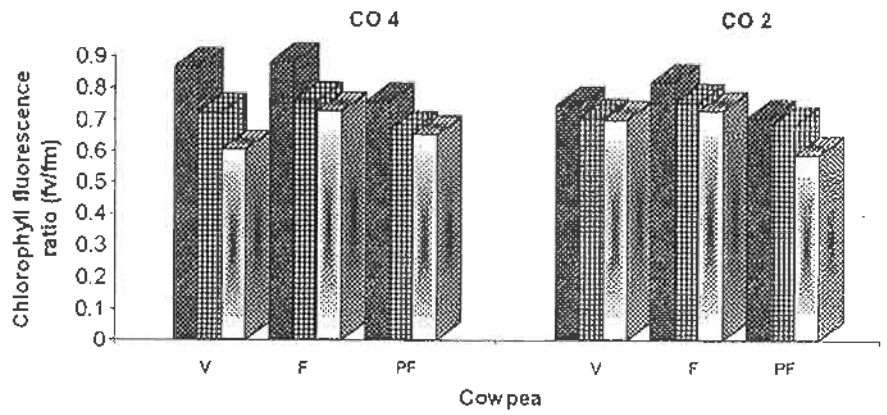
Green gram

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Table 25. Effect of flood stress on chlorophyll fluorescence ratio (fv / fm) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control		Flooding for	Control		Flooding for	Control		Flooding for					
	5 days	10 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days					
Cowpea	CO 4	0.864	0.721	0.604	0.728	0.880	0.758	0.726	0.788	0.757	0.667	0.653	0.692	0.737
	CO 2	0.745	0.708	0.700	0.718	0.818	0.755	0.728	0.767	0.706	0.683	0.589	0.659	0.715
	Mean	0.804	0.715	0.652	0.724	0.849	0.757	0.727	0.778	0.732	0.675	0.621	0.676	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.002	0.003	0.003		0.005	0.006	0.005		0.008				
Soybean	CO 1	0.708	0.626	0.611	0.648	0.869	0.779	0.717	0.789	0.782	0.647	0.608	0.679	0.705
	CO 2	0.740	0.645	0.621	0.669	0.891	0.812	0.743	0.815	0.772	0.663	0.637	0.691	0.725
	Mean	0.724	0.635	0.616	0.659	0.880	0.795	0.731	0.802	0.757	0.655	0.622	0.685	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.003	0.003	0.003		0.005	0.006	0.005		0.009				
Green gram	CO 4	0.719	0.690	0.608	0.672	0.801	0.782	0.754	0.779	0.750	0.624	0.602	0.658	0.703
	VBN 1	0.699	0.648	0.598	0.648	0.836	0.757	0.737	0.777	0.670	0.638	0.632	0.646	0.691
	Mean	0.709	0.669	0.603	0.660	0.819	0.770	0.746	0.778	0.710	0.631	0.617	0.652	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.002	0.003	0.003		0.004	0.005	0.005		0.008				

Fig. 12. Effect of flood stress on Chlorophyll Fluorescence ratio (fv / fm)



■ 0 days ■ 5 days ■ 10 days

V - Vegetative; F - Flowering; PF - Pod Filling

compared to shorter duration of flooding (5 days) at vegetative stage followed by pod filling and flowering stage. Regarding growth stages pod-filling stage recorded lowest means value of fv/fm ratio as compared to vegetative and pods filling stages. All interaction effect was significant.

4.2.8. Biochemical mechanism of flooding tolerance

4.2.8.1. Leaf soluble proteins (mg g⁻¹) (Table 26)

Cowpea

The leaf soluble protein increased steadily from the vegetative stage to flowering stage and thereafter declined towards pod filling. The two varieties differed significantly at all growth stages. The variety CO 4 showed higher soluble protein content over the variety CO 2. The increase was 7.7 per cent. Flooding treatments decreases leaf soluble protein content at all growth stages. The irrespective of the varieties, the treatments showed significant difference. The longer duration of flooding (10 days) recorded more reduction in soluble protein (28.4%) as compared to 5 days flooding (15.8%) at pod filling stages. The effect of 5 days flooding was harmless. Among the growth stages, flowering stage recorded higher soluble protein content (13.45) as followed by vegetative stage (11.49) and pod filling stage (10.58). Interaction between the varieties and stages, varieties and treatments and stages were significant.

Soybean

The data on the soluble protein content was estimated at three stages of growth. The soluble protein was highest in the flowering stage (9.86) and lowest in pod development (7.92). The varieties differed significantly for soluble protein content. The variety CO 1 (9.20) recorded higher mean value of soluble protein as compared to CO 2 (6.95). Flood treatments significantly decreases leaf soluble protein content in both varieties. Among the treatments, flooding for 10 days decreases leaf soluble protein content drastically at both flowering and pod filling stages. Comparatively flooding for 10 days had deleterious effect on soluble protein than 5 days flooding. Regarding growth stages, flowering and pod filling growth stages

Table 26. Effect of flood stress on leaf soluble protein (mg g⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean					
	Control	Flooding for		Control	Flooding for		Control	Flooding for							
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean		
Cowpea	CO 4	13.91	12.46	10.52	12.30	15.56	12.64	11.95	13.38	12.21	11.37	10.28	11.28	12.32	
	CO 2	12.62	10.72	8.71	10.68	14.95	14.10	11.50	13.52	12.63	9.53	7.49	9.88	11.36	
	Mean	13.27	11.59	9.62	11.49	15.26	13.37	11.73	13.45	12.42	10.45	8.89	10.58		
CD		V	T	S		VXT	TX	S	VXS	VXTXS					
		0.463	0.568	0.568		0.803	0.983	0.803		1.391					
Soybean	CO 1	8.59	5.79	5.21	6.53	14.75	12.15	7.88	11.59	12.47	8.68	7.29	9.48	7.92	
	CO 2	6.47	7.28	5.35	6.36	10.19	8.12	6.07	8.13	8.28	6.48	4.29	6.35		
	Mean	7.53	6.54	5.29	6.45	12.47	10.14	6.98	9.86	10.38	7.58	5.79	7.92		
CD		V	T	S		VXT	TX	S	VXS	VXTXS					
		0.476	0.583	0.583		0.825	1.009	0.825		1.428					
Green gram	CO 4	18.18	15.30	13.39	15.69	20.82	15.73	12.67	16.41	15.80	13.28	11.48	13.52	15.21	
	VBN 1	17.60	14.46	12.62	14.89	21.28	15.43	14.01	16.91	13.49	12.19	10.38	12.02	14.61	
	Mean	17.89	14.88	13.11	15.29	21.05	15.58	13.34	16.66	13.65	12.74	10.93	12.77		
CD		V	T	S		VXT	TX	S	VXS	VXTXS					
		0.464	0.568	0.568		0.803	0.984	0.803		1.392					

were more susceptible to flooding treatments compared to vegetative stage. Interaction effects were found to be significant.

Green gram

Significant variation between the varieties for leaf soluble protein content. The variety CO 4 (15.21) recorded highest mean value of leaf soluble protein compared to VBN 1 (14.61). Flooding treatments lower the protein content of leaves. Among the flooding treatments, higher duration of flooding (10 days) recorded higher reduction protein content of leaves (36.6%) as compared to 5 days of flooding (25.9%) at flowering stages. At different growth stages, flowering stage highly sensitive to flooding followed by vegetative and pods filling stages. Interaction between the treatments and stages, varieties and stages were found to be significant.

4.2.8.2 Root anaerobic protein (mg. g⁻¹) (Table 27; Fig. 13)

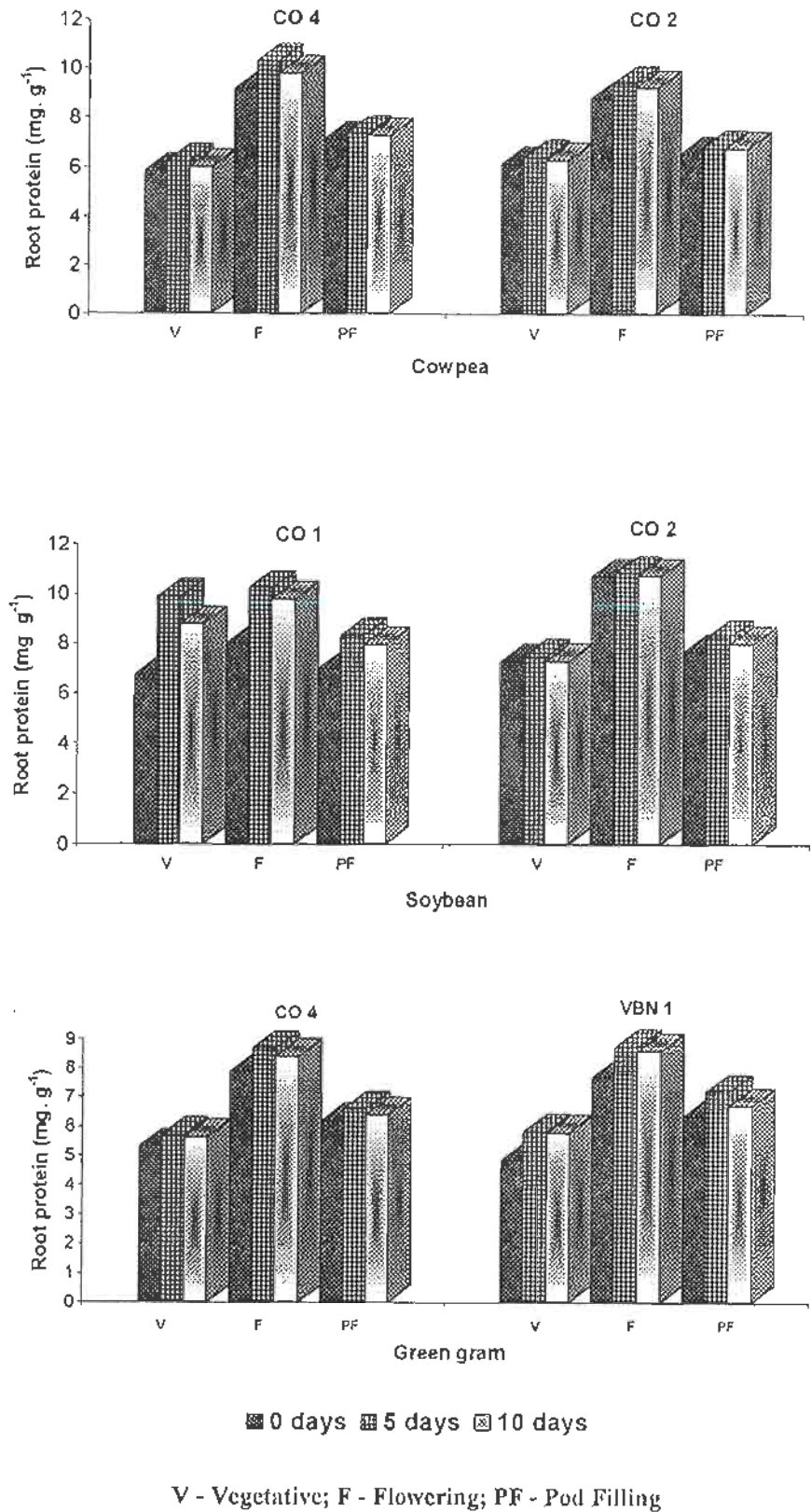
Cowpea

The data on root anaerobic protein indicated that increase was due to flooding at all stages. No significant difference was found between varieties and treatments. The variety CO 4 recorded higher accumulation of root anaerobic protein compared to CO 2 at flowering stage. The anaerobic root protein was significantly decreased with the increased duration of flooding (5 days to 10 days) at all stages. However, flooding for 5 days recorded higher content of anaerobic root protein compared to 10 days flooding at flowering stages. At different growth stages, flowering stage (9.46) had more content of root protein as compared to pod filling (6.99) and vegetative (6.16) stages. Interaction effects were non significant.

Soybean

The root proteins increase up to flowering and thereafter it declined to pod filling stage. Significant difference was found between the varieties. The variety CO 1 (8.5) recorded highest mean value of root proteins as compared to CO 2 (8.8). Flooding treatment significantly increases root anaerobic protein at all growth stages. Among the different flooding treatments, 5 days flooding increases root proteins upto 12.6 per cent as compared to 10 days flooding (9.7%) over control at flowering stage.

Fig. 13. Effect of flood stress on Root protein (mg g^{-1})



The effect of 10 days flooding was less. Among the different growth stages, the flowering stages exhibit a higher content of root proteins compared to pod filling and vegetative stages. The interaction between variety and treatment showed the CO 1 (9.88) recorded higher root protein content than CO 2 (7.47) under 5 days of flooding. Interaction between variety and stage revealed the CO 2 (10.79) recorded higher content of protein than CO 1 (9.32) at flowering stages.

Green gram

Similar to cowpea and soybean, the green gram root anaerobic proteins increase upto flowering and there after it declined. No significant differences were found between the varieties (CO 4 and VBN 1). The mean value of root proteins of CO 4 on par with the variety VBN 1. Flooding increases the root anaerobic proteins. Among different treatments, flooding for 5 day accumulates more anaerobic root protein (13.45) as compared to 10 days flooding (8.52) over to control (7.78) at vegetative stage. At different growth stages, flowering stage accumulates high content of root proteins compared to other growth stages under all flood treatments. Interaction effects were found to be non-significant.

4.2.8.3. Carbohydrates fractions

4.2.8.3.1. Starch (mg g^{-1}) (Table 28)

Cowpea

Varieties tested were affected in leaf starch content both the duration and stage of flooding. Flooding significantly induced starch content of leaves as compared to control. The variety CO 2 (41) recorded higher mean values of starch as compared to CO 4 (39). Both the varieties had lower accumulation of starch under flooding for 10 days compared to 5 days of flooding. Under 10 days of flooding, the variety (CO 2) had higher reduction of starch compared to CO 4. Among the treatments, flooding for 10 days (21.4) accumulates lower content of starch compared to 5 days flooding (26.1) over control (29.6) at vegetative stage. Under different growth stages, the pod filling accumulates more starch content followed by flowering and vegetative stage. Interaction between the varieties and stages were significantly showed the

highest starch content recorded in CO 2 (42.5) and lowest in CO 4 (37.1) at flowering stage.

Soybean

Significant difference was found between the varieties, treatments and stages for starch. The overall means values of starch were showed higher in CO 1 (51.5) than CO 2 (49.3). Flooding stress reduces starch content at all the stages. Among the flooding treatment, flooding for 10 days recorded higher reduction of starch content compared to 5 days flooding (16%) over control at vegetative stage. Under 10 days flooding, the variety CO 2 (38.4%) had more reduction in starch content compared to CO 1 (30.3%) at vegetative stage. Similar to cowpea regarding growth stages, the pod filling accumulates more starch content followed by flowering and vegetative filling. Interaction between varieties and treatments were found to be significant revealed that 10 days of flooding caused reduction of 27% and 23% in CO 1 and CO 2 respectively.

Green gram

Similar to soybean, the significant difference was noticed in varieties, treatments and stages. The mean values of starch were almost nearer in both the varieties. Under flooding treatments both the varieties (CO 4 and VBN 1) had lower content of starch. Among the treatments, flooding for 10 days recorded lower content of starch compared to 5 days recorded lower content of starch compared to 5 days flooding at all growth stages. However the maximum starch content reduction was noticed in flowering stage followed by vegetative and pod filling stages. Under 10 days of flooding, the variety VBN 1 (33.8%) showed more reduction of starch content compared to CO 4 (27.7%) at flowering stage. Regarding growth stages as like that of cowpea and soybean. Interaction effects were found to be significant.

4.2.8.3.2. Total carbohydrates (mg g⁻¹) (Table 29)

Cowpea

There was significant variation in total carbohydrates content among the varieties. The total carbohydrate content (averaging over all stages of stress and control) was higher in CO 4 (23.4) than CO 2 (21.8). Flooding significantly decreased

Table 29. Effect of flood stress on total carbohydrates content (mg g⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	15.17	13.13	12.00	13.43	29.43	27.70	25.10	27.41	32.80	28.33	26.90	29.34	23.39
	CO 2	13.33	11.00	9.70	11.34	31.33	28.03	23.00	27.46	28.73	27.90	23.03	26.56	
	Mean	14.25	12.07	10.85	12.39	30.38	27.87	24.05	27.43	30.77	28.12	24.97	27.95	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.899	1.101	1.101		1.558	1.907	1.558		2.701				
Soybean	CO 1	13.93	11.93	11.00	12.29	27.83	26.60	24.70	26.38	26.60	25.33	24.73	25.56	21.41
	CO 2	12.43	11.73	10.57	11.58	24.53	22.93	20.40	22.62	24.70	22.70	20.33	22.58	18.93
	Mean	13.18	11.83	10.78	11.93	26.18	24.77	22.55	24.50	25.65	24.02	22.53	24.07	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.924	1.132	1.132		1.601	1.962	1.602		2.774				
Green gram	CO 4	11.53	9.83	8.90	10.09	23.03	21.03	19.10	21.06	22.67	21.00	19.20	20.01	17.48
	VBN 1	10.0	9.40	8.37	9.37	20.83	20.03	16.90	19.26	21.63	20.60	16.93	19.72	16.11
	Mean	10.93	9.62	8.63	9.73	21.93	20.53	18.00	20.16	22.15	20.80	18.07	19.87	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.908	1.112	1.112		1.573	1.923	1.573		2.725				

the level of total carbohydrates over control. Among the varieties, CO 2 (27.2%) recorded maximum reduction of total carbohydrate content compared to CO 4 (20.9%) on 10 days of flooding at vegetative stage. In the treatments, flooding for 10 days (10.85) had lower in total carbohydrate content compared to 5 days flooding (12.07) over control (14.25). Irrespective of the treatments, both the flowering and pod filling stages showed higher content of total carbohydrates followed by vegetative stage. Interaction effects were found to be significant.

Soybean

Total carbohydrate content significantly differed among varieties with the highest over all mean value of 21.41 in CO 1 and the lowest in CO 2 (18.93). Flooding decreased the accumulation of total carbohydrate content significantly. Among the genotypes, CO 1 accumulated the lesser percentage of total carbohydrate (21) compared to CO 2 (15) on 10 days of flooding at vegetative stage. Among the treatments, the flooding for 10 days (10.78) recorded lower content of total carbohydrates compared to 5 days flooding (11.83) over control (13.18) at vegetative stage. At different stages, total carbohydrates accumulated on par at flowering and pod filling stages. Interaction between treatment and stage, variety and stage were significant.

Green gram

The genotypes differed significantly in their total carbohydrate content. The over all mean of total carbohydrate content was highest in CO 4 (17.48) and lowest in VBN 1 (16.11). Flooding significantly decreases the total carbohydrate content between the varieties. Among the varieties CO 4 recorded higher reduction of total carbohydrates (22.8%) compared to VBN 1 (18.7%) on 10 days flooding at vegetative stage. Irrespective of the varieties, longer duration of flooding accumulated lower content of total carbohydrates compared to shorter duration of flooding at vegetative stages. At different stages, both flowering and pod filling recorded higher content of carbohydrates compared to vegetative stage. Interaction between treatments and stages was significant.

4.2.8.4. Nitrate Reductase (NRase) (μ moles NO_2 produced $\text{g}^{-1} \text{h}^{-1}$) (Table 30)

Cowpea

The activity of nitrate reductase is an important enzyme in nitrate assimilation. The higher enzyme activity was recorded at flowering stage. Significant difference exist between the varieties for nitrate reductase. Among the varieties the variety CO 4 (17.03) possessed higher enzyme activity over the other variety CO 2 (15.48). Irrespective of the varieties, the treatments showed nonsignificant difference at all growth stages. The treatment flooding for 10 days registered lower enzyme activity (9.33) compared to flooding for 5 days (10.98) over control (12.86) at vegetative stage. Among the stages, flowering registered higher enzyme activity followed by vegetative and flowering stages. Interaction between varieties and stages were significantly showing that the CO 4 (31.11) recorded higher NRase activity than CO 2 (24.89) at flowering stage.

Soybean

Significant reduction in NRase activity was noted due to flooding treatments. The genotypes varied significantly in their NRase activity at various stages of flooding duration. The variety CO 2 (10.40) recorded higher activity of enzyme as compared to CO 1 (9.02). Among the different flooding treatments, the flooding for 10 days had higher reduction in activity of enzymes (35%) as compared to 5 days flooding (18%) over control at vegetative stage. The variety CO 2 recorded lower activity of enzymes under 10 days flooding compared to CO 1 which is very nearer to CO 2 at vegetative stage. Significant difference between the stages was also significant. Flowering stage recorded higher enzyme activity followed by pod filling and vegetative stage. Interaction between treatment and stage, variety and stage were significant.

Green gram

Significant difference between the varieties, treatments and stages for NRase activity. The variety VBN 1 (14.76) recorded higher mean value of NRase activity compared to CO 4 (12.03). Flooding treatments decreases the NRase activity at all growth stages. Irrespective of the varieties, the flooding for 10 days (28.9%) recorded

Table 30. Effect of flood stress on leaf nitrate reductase activity (μ moles NO_2 produced $\text{g}^{-1} \text{h}^{-1}$) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	12.59	10.87	8.84	10.76	32.58	31.30	29.46	31.11	9.70	9.39	8.59	9.22	17.03
	CO 2	13.14	11.09	9.83	11.35	30.02	25.71	18.93	24.89	10.62	10.38	9.64	10.21	15.48
	Mean	12.86	10.98	9.33	11.06	31.30	28.51	24.20	28.00	10.16	9.88	9.16	9.72	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	6.047	7.406	7.406		10.474	12.828	10.474		18.142				
Soybean	CO 1	4.26	3.09	2.78	3.38	15.33	12.17	10.00	12.50	12.53	11.39	9.63	11.18	9.02
	CO 2	5.55	5.01	3.60	4.72	21.16	18.98	16.36	18.83	8.87	7.60	6.51	10.40	10.40
	Mean	4.91	4.05	3.10	4.05	18.24	15.58	13.18	15.67	10.70	9.50	8.01	9.42	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.435	0.533	0.533		0.754	0.923	0.754		1.754				
Green gram	CO 4	9.20	7.90	7.52	8.21	20.18	17.51	16.16	17.95	12.53	9.63	7.61	9.92	12.03
	VBN 1	12.65	11.53	10.03	11.40	25.18	21.60	19.66	22.15	11.72	10.83	9.62	10.72	14.76
	Mean	10.92	9.72	8.77	9.80	22.68	19.55	17.91	20.05	12.12	10.23	8.61	10.32	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.404	0.495	0.495		0.699	0.857	0.699		1.212				

more reduction of enzyme activity compared to 5 days (15.5%) flooding over control at pod filling stages. The variety CO 4 recorded higher reduction of NRase activity at pod filling stage and VBN 1 at flowering stages due to 10 days of flooding. Regarding growth stages, flowering recorded higher activity of NRase followed by pod filling and vegetative stages. Interaction between treatments and stages revealed that flooding for 10 days recorded higher reduction in activity of NRase (21.0%) compared to 5 days flooding (13.8%) at flowering stage.

4.2.9. Ethanol fermentation enzymes

4.2.9.1. Alcohol Dehydrogenase activity (ADH) (μ moles of NADH oxidised $\text{min}^{-1} \text{mg}^{-1}$) (Table 31)

Cowpea

The data on alcohol dehydrogenase (ADH) activity was significantly increased by flooding at all stages. Among the flooding treatments, flooding for 5 days (0.331) had higher activity of ADH compared to 10 days flooding (0.169) over control (0.206) at flowering stage. The data also indicated significant variation between varieties in respect of their tolerance. Variety CO 4 recorded higher activity of ADH (62%) compared to CO 2 (60%) due to 5 days flooding at flowering stage. The overall means value of CO 4 recorded more ADH activity than CO 2. Irrespective of the treatments, the flowering stage recorded high activity of ADH followed by pod filling and vegetative stage. Irrespective of the stages, the flooding for 5 days observed higher activity of ADH as compared to 10 days flooding at all growth stages. Interaction between treatment and stage were significantly showed that higher activity of ADH was noticed in 5 days flooding over control at flowering as followed by pod filling and vegetative stage. Interaction between flooding treatments, varieties and stages were also significant.

Soybean

The data on alcohol dehydrogenase activity was significantly increased upto flowering stage and thereafter it declined. No significant difference found between the varieties. All flooding treatments increase the ADH activity. Among flooding

Table 31. Effect of flood stress on Alcohol Dehydrogenase (ADH) Activity (μ moles NADH oxidized $\text{min}^{-1} \text{mg}^{-1}$ of sample) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean			
	Control	Flooding for 10 days		Control	Flooding for 10 days		Control	Flooding for 10 days					
		5 days	Mean		5 days	Mean		5 days	Mean				
Cowpea	CO 4	0.125	0.188	0.166	0.159	0.215	0.343	0.185	0.248	0.245	0.202	0.210	0.206
	CO 2	0.138	0.176	0.145	0.153	0.198	0.320	0.153	0.223	0.226	0.212	0.197	0.191
	Mean	0.131	0.182	0.155	0.156	0.206	0.331	0.169	0.235	0.235	0.207	0.204	
CD		V	T	S		VXT	TXS	VXS		VXTXS			
		0.006	0.007	0.007		0.010	0.012	0.010		0.017			
Soybean	CO 1	0.118	0.189	0.161	0.156	0.185	0.258	0.126	0.189	0.210	0.187	0.174	0.173
	CO 2	0.123	0.195	0.150	0.156	0.199	0.288	0.138	0.208	0.203	0.158	0.166	0.177
	Mean	0.120	0.192	0.155	0.156	0.192	0.273	0.138	0.199	0.207	0.173	0.170	
CD		V	T	S		VXT	TXS	VXS		VXTXS			
		0.006	0.007	0.007		0.012	0.010	0.010		0.016			
Green gram	CO 4	0.132	0.176	0.148	0.152	0.206	0.250	0.165	0.207	0.236	0.212	0.204	0.188
	VBN 1	0.126	0.198	0.165	0.163	0.216	0.303	0.179	0.232	0.220	0.200	0.199	0.198
	Mean	0.129	0.189	0.156	0.157	0.212	0.276	0.172	0.220	0.228	0.206	0.202	
CD		V	T	S		VXT	TXS	VXS		VXTXS			
		0.006	0.007	0.007		0.010	0.012	0.010		0.017			

duration, flooding for 5 days increased ADH activity compared to 10 days flooding at all growth stages. Flooding for 10 days recorded more activity of ADH compared to control during all growth stages. Among the stages the flowering recorded higher activity of ADH followed by pod filling and vegetative stage. The interaction effects V x T, T x S and V x S were significant.

Green gram

Data on ADH activity showed an increasing trend under flooding conditions at all the stages of plant. The mean value of ADH activity recorded higher in VBN 1 than CO 4. Highly significant variation was noticed between treatments with the activity of enzyme ADH. In flooding treatments, 5 days for flooding (0.189 recorded higher activity of enzymes compared to 10 days flooding over control (0.129) at vegetative stage. The variety VBN 1 recorded higher percentage of activity was 57.1 as compared to CO 4 (33.3%) over control on 5 days of flooding at vegetative stage. Among the stages, vegetative stage recorded lesser mean value of ADH compared to pod filling and flowering stages. There was a significant difference between varieties and stages. The T x S, V x S and V x T x S interaction were also significant.

4.2.9.2. Pyruvate Decarboxylase activity (PDC) (enzyme units g⁻¹ fresh wt.) (Table 32)

Cowpea

Treatments and stages exhibited significant difference in PDC activity. The mean value of pyruvate decarboxylase (PDC) activity was slightly higher in CO 2 (0.192) than CO 4 (0.140). No significant difference between the varieties. In flooding treatments, shorter duration of flooding (5 days) recorded higher PDC activity compared to longer duration of flooding (10 days) over control at vegetative stages. Among the different stages, pod-filling stages recorded higher activity of PDC followed by flowering and vegetative stage. Interaction between treatments and stages were significantly showed flooding for 5 days recorded higher PDC (0.119) activity as compared to control (0.054) at vegetative stage.

Soybean

There was significant variation between varieties, treatments and stages. The mean value of PDC was higher in CO 2 (0.190) as compared to CO 1 (0.179). Flooding stress increases PDC activity in both the varieties significantly. In flooding treatments, flooding for 5 days (0.149) increases PDC activity compared to flooding for 10 days (0.120) over control (0.069) at vegetative stage. Under 5 days of flooding, the PDC activity was higher in CO 2 (0.168) compared to CO 1 (0.130) at vegetative stage. Among the stages, pod filling recorded higher activity of PDC followed by flowering and vegetative stage. Interaction between treatment and stage were significant.

Green gram

There was no significant difference between the varieties and treatments. The overall mean value of PDC activity was higher in CO 4 (0.174) compared to VBN 1 (0.158). Flood treatments increases the PDC activity in both the varieties. Under 5 days flooding, both the variety VBN 1 and CO 4 recorded higher activity of PDC over control. Among the treatments, flooding for 5 days showed higher activity of PDC (0.113) as compared to 10 days flooding (0.097) over control (0.043) at vegetative stage. At different stages, flowering showed higher activity of PDC followed by pod filling and vegetative stages. Interaction effects were non-significant.

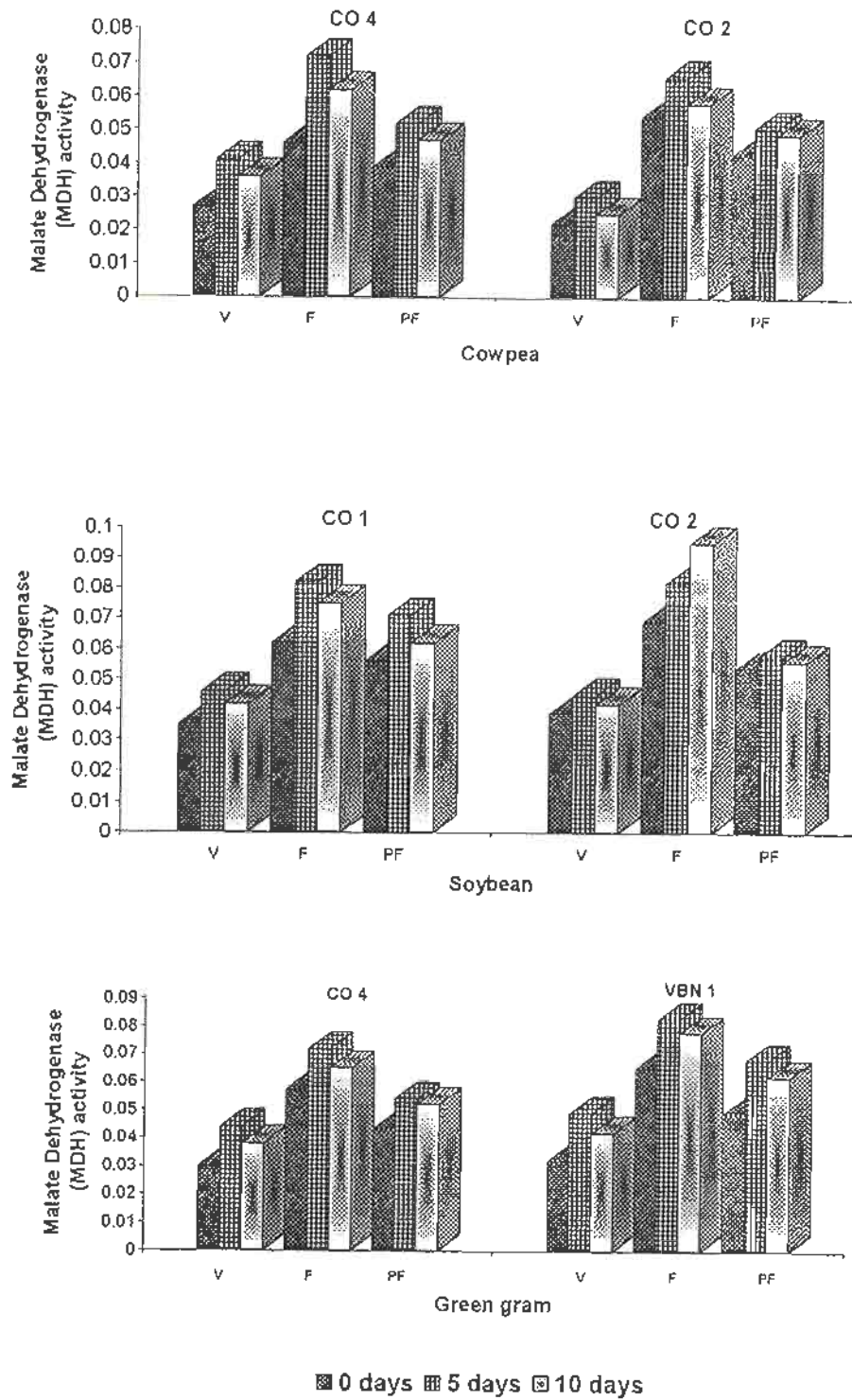
4.2.10. Alternative fermentation pathway enzyme

4.2.10.1. Malate Dehydrogenase (MDH) activity (μ moles NADH oxidised min^{-1} ml of extract) (Table 33; Fig. 14)

Cowpea

Significant difference in MDH activity was exhibited by varieties under flooding conditions at all growth stages. The mean values of MDH activity recorded higher in roots of CO 4 (0.048) compared to CO 2 (0.045). Under different flooding treatments, shorter duration of flooding (5 days) recorded higher activity in MDH compared to longer duration of flooding (10 days) at all growth stages. Irrespective of the treatments, the flowering stage recorded higher activity of MDH as compared to

Fig. 14. Effect of flood stress on Malate Dehydrogenase (MDH) activity (μ moles NADH oxidized $\text{min}^{-1} \text{mg}^{-1}$ of extract)



V - Vegetative; F - Flowering; PF - Pod Filling

other stages. The variety CO 4 recorded more activity of MDH under all flooding treatments as compared to variety CO 2. Regarding growth stages the flowering recorded higher activity of MDH followed by pod filling and vegetative stage. All interaction effects were significant except V x T x S.

Soybean

The data on MDH activity were significantly difference between the treatments and stages. But no significant difference between the varieties. Flooding increases the MDH activity at all stages. However, the variety CO 1 (0.082) recorded higher activity of MDH than CO 2 (0.073) at flowering stage. Among the treatments, short duration of flooding (5 days) recorded higher per cent of MDH activity (35.3) compared to 10 days flooding (21.5) at flowering stage. Different growth stages the mean values of MDH activity on par with each other at vegetative and flowering stages. Interaction between T x S, V x S and V x T x S were significant.

Green gram

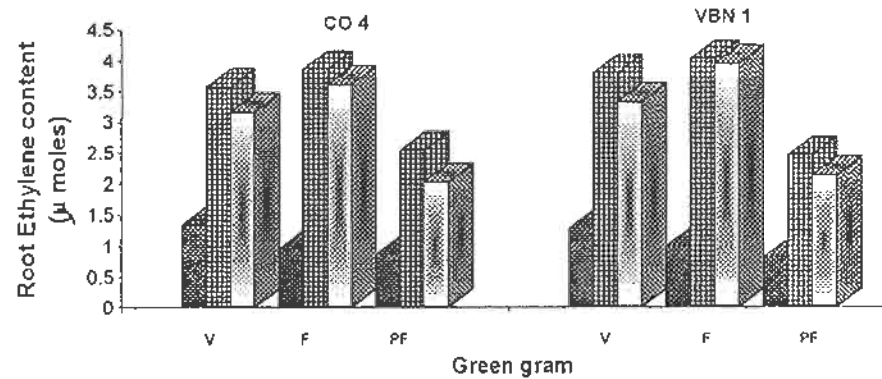
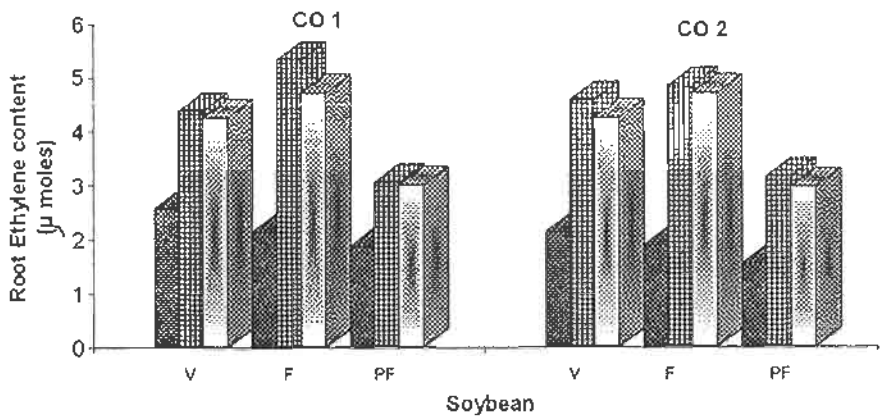
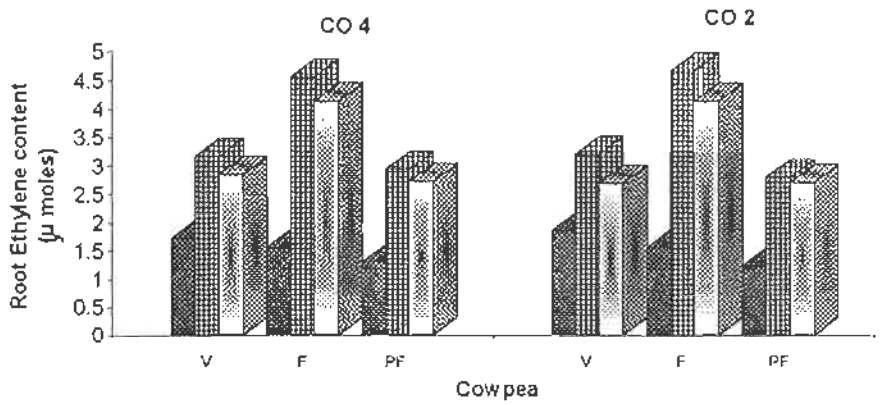
Varieties, treatments and stages exhibited significant difference in MDH activity. The mean values of root MDH activity were higher in CO 4 (0.059) compared to VBN 1 (0.050). Flooding increases MDH activity at all the stages. Among the different flood treatments, higher activity of MDH was noticed in 5 days of flooding (0.047) compared to 10 days of flooding (0.041) at vegetative stage. Irrespective of the treatment, the flowering stage recorded the more activity of MDH (0.070) in comparison with pod filling (0.054) and vegetative (0.041) stages. Interaction between variety and stage was significantly recorded higher MDH activity in CO 4 (0.075) than VBN 1 (0.065) at flowering.

4.2.11. Ethylene (μ moles) (Table 34; Fig. 15)

Cowpea

The data on ethylene concentration in roots showed a significant difference between treatment and stages. No significant difference between the varieties for ethylene. Flooding treatments increases ethylene in both the varieties (CO 4 and CO 2). Among the flooding treatments, flooding for 5 days releases more ethylene in

Fig. 15. Effect of flood stress on Root Ethylene content (μ moles)



■ 0 days ■ 5 days ■ 10 days

V - Vegetative; F - Flowering; PF - Pod Filling

comparison with flooding for 10 days at all the stages. On over all mean bases, variety CO 4 was on par with CO 2 for ethylene concentration. Regarding growth stages, flowering stage (3.447) recorded higher concentration of ethylene followed by vegetative (2.591) and pod filling stage (2.296). Interactions between treatments and stage revealed that higher concentration of ethylene released over control by 5 days of flooding at flowering stage.

Soybean

Significant difference was found to between the varieties, treatments and stages. Varieties used were released more concentration of ethylene both by the duration and stages of flooding. The overall mean basis, variety CO 1 (3.475) were recorded higher concentration of ethylene in comparison with CO 2 (3.338) variety. Among the flooding treatments, similar to cowpea the 5 days flooding (4.619) recorded higher concentration of ethylene as compared to 10 days flooding (4.146) over control (1.577) at flowering stage. Under 5 days of flooding, variety CO 1 had maximum concentration of ethylene followed by the variety CO 2 at all growth stages. Among the growth stages, Pod filling stage recorded lower concentration of ethylene followed by vegetative and flowering stages. Interaction between V x T, T x S, V x S and V x T x S were significant.

Green gram

Similar to green gram, the difference between the varieties, treatments and stages were significant. The overall mean of root ethylene concentration was higher in VBN 1 (2.523) compared to CO 4 (2.434). Flooding treatments increases ethylene concentration in both the varieties. Among the different days of flooding the variety VBN 1 recorded higher concentration of ethylene followed (4.018) by the variety CO 4 (3.859) under 5 days of flooding at flowering stage. Irrespective of the varieties 5 days flooding recorded more concentration of ethylene in comparison with the 10 days of flooding at all the stages. With the respect of growth stages, flowering stages showed higher amount of ethylene followed by vegetative and pod filling stages. Interaction between V x T and T x S and V x S were significant.

4.2.12. Free radical scavenging enzymes

4.3.12.1. Peroxidase (enzyme units $l^{-1} h^{-1}$) (Table 35)

Cowpea

The mean peroxidase activity was significantly higher in CO 2 (108) and on par with CO 4 (107). Flood stressed plants recorded significantly higher peroxidase activity as compared to control. Among the treatments, flooding for 10 days recorded higher activity of peroxidase (94.86) compared to 5 days (112.5) of flooding over control (110.1) at flowering stage. At different stage, pod-filling stage had more activity of peroxidase (137) followed by flowering (106) and vegetative stage (80), respectively. Interaction between treatment and stage showed that higher activity of peroxidase by 10 days of flooding at flowering stage. Interaction between variety and stage found that peroxidase activity of CO 2 (144) was significantly less as compared to CO 4 (130) at pod filling stages.

Soybean

Peroxidase activity was significantly higher due to flooding treatment and the activity was recorded in 10 days of flooding (106) compared to control (82.4) at flowering stage. The effect of 5 days of flooding was less. Cultivars did not differ significantly in peroxidase activity. Interaction between variety and treatment showed that higher activity in peroxidase by 103 in CO 1 than CO 2 (100) due to flooding for 10 days. Interaction between treatments and stages revealed that higher activity of peroxidase was noticed in 10 days of flooding (106) over control (82) at flowering stage. Interaction between variety and stage indicated that higher activity of peroxidase was noted in CO 2 (123) than CO 1 (119) at flowering stage. Interaction between variety and treatment and stage were also significant.

Green gram

Flooding increased the peroxidase activity significantly all stages. There is no significant difference between the varieties and treatments. At different growth stages, pod filling recorded higher activity of peroxidase (113) as compared to flowering (91) and vegetative stage (66) respectively. At flowering stage, the flooding for 10 days recorded higher activity of peroxidase (103) compared to 5 days (93) flooding

Table 35. Effect of flood stress on peroxidase activity (enzyme units l⁻¹ h⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	76.48	82.38	86.07	81.64	98.30	112.65	116.03	108.99	126.96	129.09	133.05	129.70	106.78
	CO 2	72.99	80.61	81.63	74.41	91.43	107.51	108.89	102.61	141.54	143.66	146.68	143.96	108.33
	Mean	74.75	81.49	83.85	80.03	94.86	110.08	112.46	105.80	134.25	136.38	139.86	136.83	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
(P=0.05)		1.099	1.345	1.345		1.903	2.331	1.903		3.296				
Soybean	CO 1	65.50	77.91	80.59	74.67	81.40	103.03	110.84	98.45	119.90	119.03	118.23	119.05	97.38
	CO 2	69.29	74.68	75.46	73.14	83.42	95.86	100.51	93.26	120.91	123.06	124.86	122.95	76.45
	Mean	67.40	76.30	78.03	73.90	82.41	99.44	105.67	95.84	120.41	121.05	121.55	120.99	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
(P=0.05)		1.270	1.555	1.555		2.200	2.604	2.200		3.811				
Green gram	CO 4	54.98	69.06	69.13	64.06	71.01	87.91	99.01	85.98	105.79	107.95	112.71	108.82	86.28
	VBN I	58.61	71.99	73.58	68.06	83.38	98.31	106.13	95.99	114.79	116.92	120.85	117.52	93.86
	Mean	56.79	70.03	71.36	66.06	77.19	93.11	102.57	90.96	110.29	112.44	111.78	113.17	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
(P=0.05)		9.289	11.377	11.377		16.091	19.707	16.091		27.870				

over control (77) respectively. The mean values of peroxidase were higher in VBN 1 (94) than CO 4 (86). Interaction effects were non-significant.

4.2.12.2. Catalase (enzyme units x 10⁴ g⁻¹ min⁻¹) (Table 36)

Cowpea

Catalase enzyme activity had an increasing trend in all the treatments through the growth stages. The enzyme activity was maximum at pod filling stage in both the varieties. Both the varieties (CO 4 and CO 2) showed significant difference in enzyme activity, where the CO 4 recorded maximum enzyme activity compared to CO 2. The per cent increase was 6.2. Among the treatments, flooding for 10 days had maximum enzyme activity (8.94) compared to 5 days of flooding (6.62) over control (4.12) at flowering stage. The variety CO 4 showed maximum activity (9.62) under 10 days of flooding compared to CO 2 (8.26) at flowering stage. Interaction between variety and treatment showed that the CO 4 recorded higher activity of catalase (8.49) than CO 2 (7.29) on 10 days of flooding. Interaction between variety and stage revealed the CO 2 recorded higher activity of catalase (6.42) than CO 4 (4.85) at vegetative stage. Other interaction effects were significant.

Soybean

Similar to that of cowpea the enzyme activity had an increasing trend in all the treatments throughout the growth stages. The enzyme activity was maximum at pod filling stage in both the varieties (CO 1 and CO 2). The mean values of catalase were higher in CO 1 than CO 2. Flooding increases the catalase activity, the variety CO 1 recorded highest activity compared to CO 2 under 10 days flooding at all stages. Among the treatments, flooding for 10 days recorded maximum activity of enzyme compared to 5 days flooding over control at pod filling stages. At different growth stages, pod filling stage recorded higher activity of enzyme followed by flowering and vegetative stages, respectively. Interaction effects between the variety and treatment showed that higher activity of catalase by 8.1 in CO 1 than CO 2 (7.7) due to 10 days of flooding. Interaction between the varieties and stages showed that the CO 1

recorded higher activity of catalase (7.38) than CO 2 (6.40) at pod filling stages. Other interaction effects were significant.

Green gram

The over all catalase activity was maximum in pod filling stage (6.47) followed by flowering stage (5.76) and vegetative stage (4.57). The mean values for the catalase activity were higher in CO 4 (5.85) than VBN 1 (5.34). Flood stress increases the catalase activity in both the varieties. Among the treatments, flooding for 10 days increases the enzyme activity compared to 5 days flooding at all the stages. The interaction between the variety and treatment showed that the variety CO 4 significantly increases the activity of enzyme compared to VBN 1 due to 10 days flooding. Interaction between the treatments and stage revealed the maximum activity recorded in 10 days of flooding (8.21) compared to 5 days of flooding (7.62) over control (3.57) at pod filling stage. The interaction between variety and stage showed that the CO 4 (6.71) noted higher activity of enzyme than VBN 1 (6.22) at pod filling stage.

4.2.12.3. Superoxide Dismutase (SOD) activity (enzyme units mg protein⁻¹ min⁻¹) (Table 37)

Cowpea

Mean SOD activity was significantly increased in stressed plants as compared to control. There is no significant variation between varieties in their SOD activity. Among the treatments, flooding for 10 days showed higher activity than 5 days of flooding at pod filling and vegetative stage. At different growth stages, pod filling recorded maximum SOD activity (4.99) as compared to flowering (4.35) and vegetative (4.22) stage, respectively. Interaction between variety and stage revealed that CO 2 recorded higher SOD activity than CO 1 at flowering stage. Interaction between VxT, TxS and VxTxS were significant.

Soybean

Mean SOD activity was significantly increased in stressed plants as compared to control. The genotypes showed significant variation in their SOD activity and CO 1

Table 37. Effect of flood stress on Superoxide Dismutase (SOD) activity (enzyme units mg protein⁻¹ min⁻¹) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for 10 days		Control	Flooding for 10 days		Control	Flooding for 10 days						
		5 days	Mean		5 days	Mean		5 days	Mean					
Cowpea	CO 4	4.20	4.27	4.36	4.28	4.33	4.29	4.34	4.32	4.90	4.93	5.03	4.95	4.52
	CO 2	4.11	4.15	4.23	4.16	4.38	4.36	4.40	4.38	5.04	5.01	5.04	5.03	4.51
	Mean	4.15	4.21	4.30	4.22	4.36	4.33	4.37	4.35	4.97	4.97	5.04	4.99	
CD (P=0.05)	V	T	S			VXT	TXS	VXS		VXTXS				
	0.035	0.043	0.043			0.062	0.075	0.062		0.170				
Soybean	CO 1	3.50	3.50	3.59	3.53	3.90	3.92	4.05	3.96	4.23	4.19	4.28	4.23	3.91
	CO 2	3.34	3.32	3.46	3.37	3.78	3.66	3.75	3.73	4.10	4.09	4.08	4.09	3.73
	Mean	3.42	3.41	3.52	3.45	3.84	3.79	3.90	3.84	4.16	4.14	4.18	4.16	
CD (P=0.05)	V	T	S			VXT	TXS	VXS		VXTXS				
	0.037	0.045	0.045			0.064	0.79	0.064		0.111				
Green gram	CO 4	2.88	2.92	3.04	2.95	4.03	4.05	4.14	4.07	4.30	4.31	4.39	4.33	3.79
	VBN 1	3.15	3.17	3.26	3.19	3.78	3.94	4.06	3.93	4.21	4.17	4.22	4.20	3.77
	Mean	3.02	3.05	3.15	3.07	3.91	4.00	4.10	4.00	4.26	4.24	4.31	4.27	
CD (P=0.05)	V	T	S			VXT	TXS	VXS		VXTXS				
	0.037	0.046	0.046			0.065	0.079	0.065		0.112				

recorded the highest mean of SOD activity (3.91) while CO 2 recorded significantly lowest mean of SOD activity (3.73). Under flooding treatments, flooding for 10 days recorded highest mean value of SOD was 3.87 as compared to 5 days of flooding was 3.78 over control 3.81. At different growth stages, pod filling recorded more activity of SOD (4.16) followed by flowering (3.84) and vegetative (3.45) stages, respectively. All interaction effects were significant.

Green gram

Similar to cowpea, there is no significant variation between varieties in their SOD activity. The mean SOD activity was significantly increased in flooding plants as compared to control. Among the treatments, flooding for 10 days recorded maximum activity (4.10) compared to flooding for 5 days (4.00) over control (3.91) at flowering stage. At different stage pod filling recorded higher activity of SOD (4.23) as compared to flowering (4.00) and vegetative stage (3.07), respectively. Interaction between variety and staged showed that the variety VBN 1 had maximum activity (3.19) as compared to CO 4 (2.95) at vegetative stage.

4.2.13. Yield and yield components

4.2.13.1. Number of flowers per plant (Numbers) (Table 38; Fig. 16)

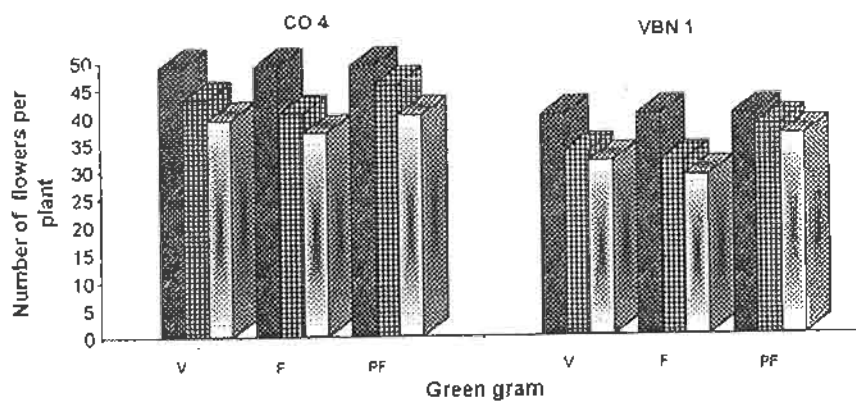
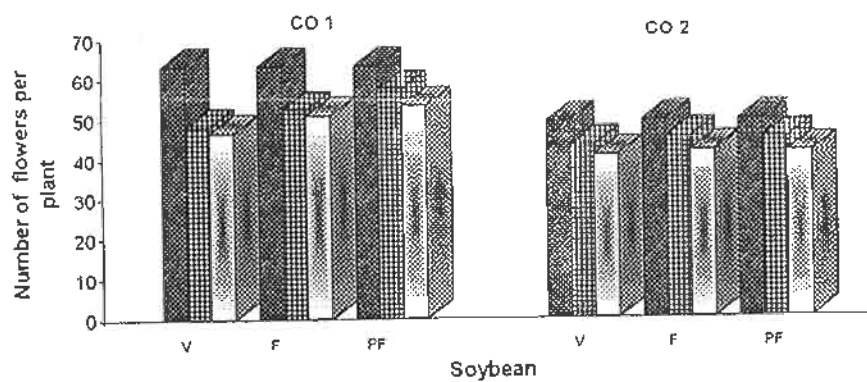
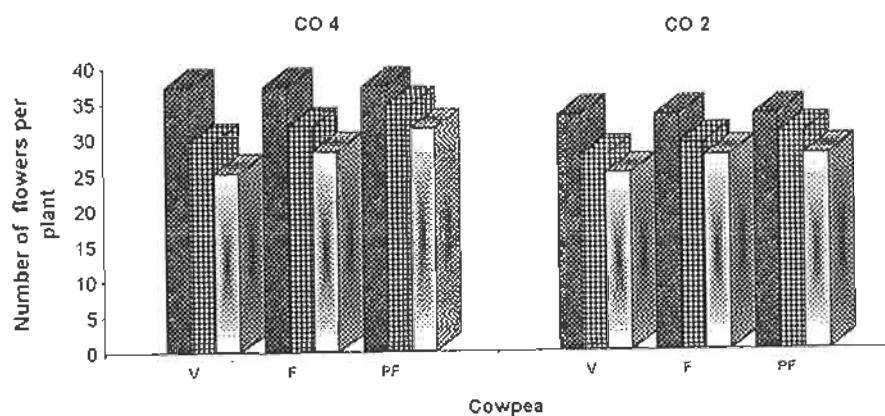
Cowpea

The overall mean number of flowers was significantly higher in CO 4 (32.4) than CO 2 (29.5). Flooding decreases the production of flowers in both the varieties. Among the flooding treatments, flooding for 10 days decreases more number of flower productions compared to 5 days flooding. At growth phases, the vegetative stages recorded less number of flower (29.5) followed by flowering (31.1) and pod filling stage (32.3). There is no significant interaction between variety and treatments. Interaction between treatments and stages significantly showed that flooding for 10 days recorded highest reduction of flower production (28.5%) compared to 5 days flooding (18.8%) at vegetative stage. Interaction between variety and stage indicated that CO 2 recorded less number of flowers (28.4) compared to CO 4 (30.6) at vegetative stage.

Table 38. Effect of flood stress on number of flowers per plant in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	37.1	29.5	25.2	30.6	37.1	31.6	28.2	32.3	37.1	34.5	31.3	34.3	32.4
	CO 2	33.0	27.4	24.9	28.4	33.0	29.1	27.3	29.8	33.0	30.4	27.4	30.2	29.5
	Mean	35.1	28.5	25.1	29.5	35.1	30.4	27.8	31.1	35.1	32.5	29.3	32.3	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		1.215	1.488	1.488		2.104	2.577	2.104		3.645				
Soybean	CO 1	63.3	48.0	46.5	52.6	63.3	52.3	50.8	55.5	63.3	57.5	53.2	58.0	55.4
	CO 2	49.2	43.0	40.6	44.3	49.2	44.9	41.6	45.3	49.2	44.7	41.3	45.1	44.9
	Mean	56.3	45.5	43.6	48.5	56.3	48.6	46.2	50.3	56.2	51.1	47.2	51.5	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		1.144	1.401	1.401		1.982	2.427	1.982		3.433				
Green gram	CO 4	49.0	43.1	39.3	43.8	49.0	40.4	36.9	42.1	49.0	45.5	40.2	44.9	43.6
	VBN 1	39.9	33.3	31.4	34.9	39.9	31.4	28.7	33.3	39.9	38.0	36.2	38.0	35.4
	Mean	44.5	38.2	35.4	39.4	44.5	35.9	32.8	37.7	44.5	41.8	38.2	41.5	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		1.032	1.064	1.264		1.787	2.189	1.787		3.096				

Fig. 16. Effect of flood stress on Number of flowers per plant



■ 0 days ▨ 5 days ▩ 10 days

Soybean

Significant variation between the varieties, treatments and stages in their flower production. The overall mean number of flowers was higher in CO 1 (55.4) than CO 2 (44.9). Among the flood treatments, flooding for 10 days decreases the production of flowers largely compared to 5 days flooding at all stages. At different growth stages, pod filling stage recorded higher number of flowers followed by flowering and vegetative stages. Interaction between the varieties and treatments revealed the variety CO 1 had higher reduction of flower production (26.5%) than CO 2 (17.5%) under 10 days flooding. Interaction between treatments and stages showed the flooding for 10 days marked higher reduction in number of flowers (22.5%) compared to 5 days flooding (19%) at vegetative stage. Interaction between varieties and stages revealed that the CO 1 (58.0) recorded higher number of flowers than CO 2 (45.1) at pod filling stage.

Green gram

There is significant difference between the varieties, treatments and stages in their flower production. Among the varieties the overall mean number of flowers was higher in CO 4 (43.6) than VBN 1 (35.4). Irrespective of the varieties, the flooding for 10 days showed higher reduction of number of flowers than 5 days flooding at all the stages. Among the stages, pod filling recorded higher number of flowers followed by flowering and vegetative stage. No significant difference between varieties and treatments. Interaction between treatments and stages revealed the 10 days for flooding recorded higher reduction of flowers (26.3%) compared to 5 days flooding (19.3%) at vegetative stages. Interaction between varieties and stages showed the variety CO 4 recorded higher number of flowered than VBN 1 at pod filling stages.

4.2.13.2. Number of pods per plant (Number) (Table 39)

Cowpea

No significant difference between the varieties and stages in their number of pods per plant. Flooding decreases the number of pods per plant. Among the treatments, flooding for 10 days reduces number of pods per plant compared to

Table 39. Effect of flood stress on number of pods per plant in pulses at different growth stages

Pulses	Vegetative stage				Flowering stage				Pod filling stage				Genotypes Mean	
	Control		Flooding for 10 days		Control		Flooding for 10 days		Control		Flooding for 10 days			
	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean	5 days	Mean		
Cowpea	CO 4	13.9	9.5	7.5	10.3	13.9	10.2	8.6	10.9	13.9	12.3	10.6	12.3	11.2
	CO 2	12.7	10.3	9.2	10.7	12.7	9.4	8.0	10.0	12.7	11.3	9.4	11.1	10.6
	Mean	13.3	9.9	8.4	10.5	13.3	9.8	8.3	10.5	13.8	11.7	10.1	11.7	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	0.351	0.427	0.427		0.601	0.734	0.601		1.042					
Soybean	CO 1	42.9	23.9	15.9	27.6	42.9	34.1	23.3	33.4	42.9	40.5	27.9	37.1	33.0
	CO 2	36.3	21.0	18.4	25.2	36.3	23.4	22.1	27.3	36.3	27.4	24.1	29.3	27.0
	Mean	39.6	22.5	17.1	26.4	39.6	28.8	22.7	30.3	39.6	34.0	26.0	33.2	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	1.517	1.858	1.858		2.628	3.129	2.628		4.553					
Green gram	CO 4	27.9	20.9	18.1	22.3	27.9	18.6	16.3	21.9	27.9	24.6	20.3	24.3	22.8
	VBN I	20.8	17.9	15.8	18.2	20.8	15.4	13.1	16.4	20.8	18.5	16.4	18.6	17.7
	Mean	24.4	19.4	17.0	20.2	24.4	17.0	14.7	19.2	24.41	21.6	18.4	21.5	
CD (P=0.05)	V	T	S		VXT	TXS	VXS		VXTXS					
	0.431	0.528	0.528		0.746	0.914	0.746		1.293					

flooding for 5 days at all stages. Interaction between treatments and stages showed the flooding for 10 days recorded higher reduction in number of pods on par with the flowering (37.6%) and vegetative stages (36.8%). The interaction between varieties and treatments, varieties and stages and V x T x S were significant.

Soybean

Significant difference between the varieties, treatments and stages. The mean number of pods per plant was higher in CO 1 (33) than CO 2 (27). Among the treatments, flooding for 10 days showed less number of pods (45.4%) compared to 5 days flooding (27.2%). At growth stages, the flowering produced less number of pod (26.4) compared to vegetative (30) and pod filling stages (33.6). Interaction between varieties and treatments showed that the CO 1 had higher reduction of number of pods (48.4%) compared to CO 2 (39.6%) under flooding for 10 days. Interaction between treatment and stage showed that flooding for 10 days recorded higher reduction of pods development (56.6%) compared to 5 days flooding (43.2%) at flowering stage. Interaction between varieties and stages indicated that the CO 1 showed higher number of pod (37.9) compared to CO 2 (29.3) at pod filling stages.

Green gram

Significant difference between the varieties and treatments. The overall means value of pod number was highest in CO 4 (22.8) than VBN 1 (17.7). Flooding reduces the number of pods in both the varieties. Among the treatments, flooding for 10 days showed lesser number of pod (16.7) as compared to 5 days flooding (19.3) over control (24.4). At different growth stages flowering recorded lesser number of pods (19.2) followed by vegetative (20.2) and pod filling (21.5). Interaction between varieties and treatments showed the CO 4 had higher reduction in pod production (34.8%) compared to VBN 1 (27.4%) under 10 days flooding. Interaction between treatment and stage showed that 10 days flooding had maximum reduction of pods development was 39.8 per cent as compared to 5 days flooding (30.3%) at flowering stage. Interaction between variety and stage revealed that variety CO 4 recorded higher number of pod (24.9) than VBN 1 (18.6) at pod filling stage.

4.2.13.3. Fertility coefficient (Table 40; Fig. 17)

Cowpea

Significant differences were observed for treatments and stages. The mean value of fertility coefficient was higher in CO 2 than CO 4. The percentage of increase was 5. Among the treatments, flooding for 10 days showed a reduction of 15.9 per cent in fertility coefficient compared to 5 days flooding (9.2%). The reduction in fertility coefficient was higher in flowering stage followed by vegetative and pod filling stages. Interaction between varieties and treatments showed the CO 4 recorded higher reduction of fertility coefficient (16.3%) compared to CO 2 (12.8%) by 10 days flooding. Interaction between treatments and stage showed that flooding for 10 days had lesser ratio of fertility coefficient compared to 5 days flooding at flowering stage. Interaction between variety and stage revealed that CO 2 recorded higher ratio of fertility coefficient than CO 4 at pod filling stage.

Soybean

Significant variations were observed for fertility coefficient among the varieties, treatments and stages. The mean value of fertility coefficient was higher in CO 1 (0.629) compared to CO 2 (0.592). Irrespective of the varieties, flooding for 10 days recorded lesser ratio of fertility coefficient (0.504) compared to 5 days flooding (0.578) over control (0.799). At different stages, pod filling recorded higher ratio of fertility coefficient (0.690) followed by flowering (0.599) and vegetative (0.543) stages. Interaction between varieties and treatments revealed that CO 1 recorded higher reduction of fertility coefficient (35%) as compared to CO 2 (31%) under 10 days flooding. Interaction between treatment and stage showed that 10 days flooding recorded lesser fertility coefficient ratio (0.395) compared to 5 days flooding (0.483) over control (0.749) at vegetative stage.

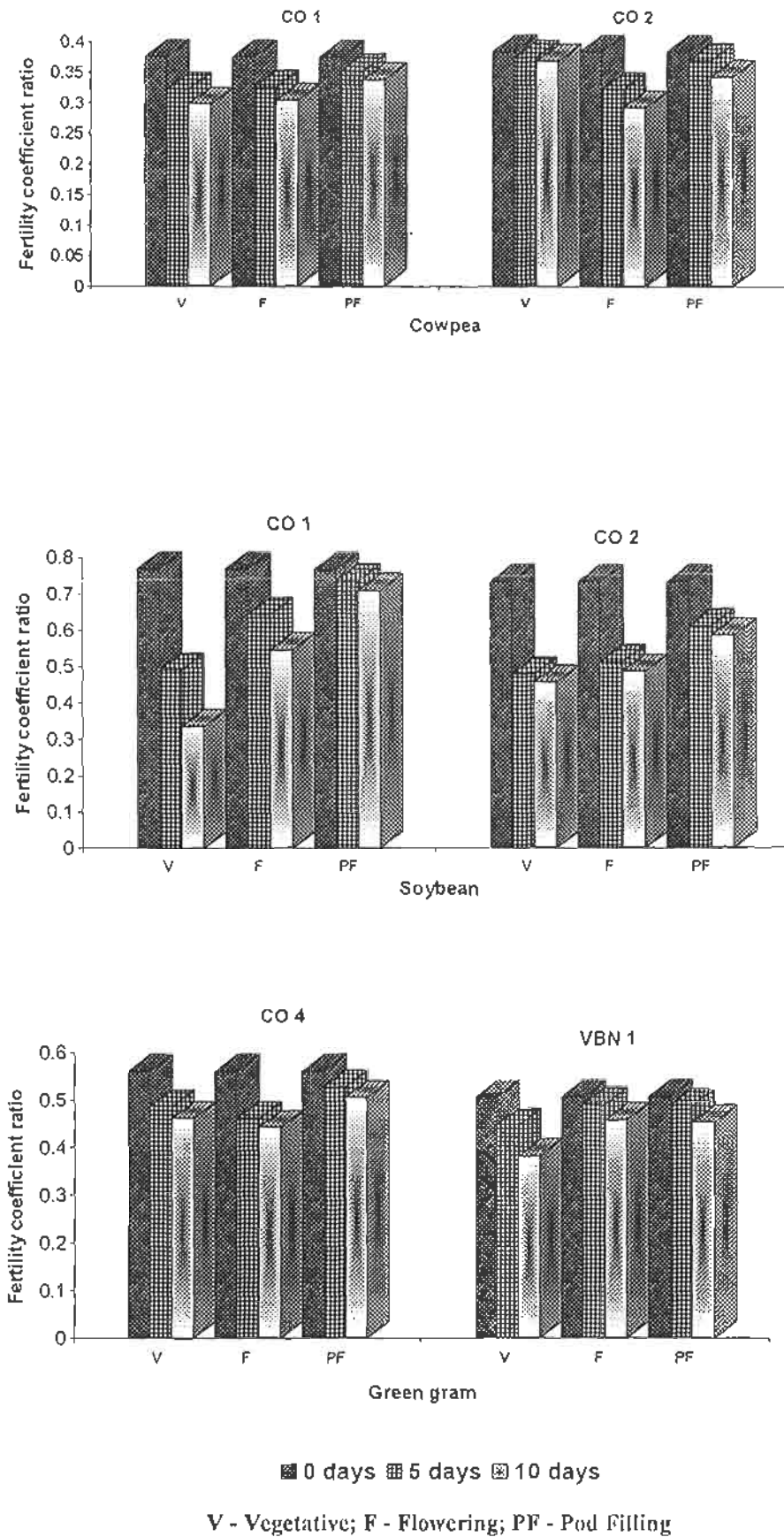
Green gram

Significant differences were observed for varieties, treatments and stages in their fertility coefficient. The mean values of fertility coefficient were higher in VBN 1 (0.506) than CO 4 (0.469). Irrespective of the treatments, the flooding for 10 days recorded lesser fertility coefficient ratio (0.459) compared to 5 days flooding

Table 40. Effect of flood stress on Fertility coefficient in pulses at different growth stages

Pulses	Vegetative stage				Flowering stage				Pod filling stage				Genotypes Mean	
	Control		Flooding for 10 days		Control		Flooding for 10 days		Control		Flooding for 10 days			
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days		
Cowpea	CO 4	0.375	0.322	0.298	0.332	0.375	0.326	0.305	0.335	0.375	0.353	0.338	0.355	0.341
	CO 2	0.384	0.375	0.369	0.376	0.384	0.323	0.293	0.333	0.384	0.371	0.344	0.366	0.358
	Mean	0.380	0.349	0.334	0.354	0.380	0.325	0.299	0.334	0.380	0.362	0.341	0.361	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.017	0.019	0.019		0.027	0.034	0.027		0.048				
Soybean	CO 1	0.766	0.489	0.334	0.530	0.766	0.642	0.544	0.621	0.766	0.737	0.709	0.737	0.629
	CO 2	0.733	0.478	0.456	0.556	0.733	0.512	0.486	0.577	0.733	0.611	0.585	0.643	0.592
	Mean	0.749	0.483	0.395	0.543	0.749	0.577	0.471	0.599	0.749	0.674	0.647	0.691	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.014	0.17	0.017		0.024	0.030	0.024		0.043				
Green gram	CO 4	0.559	0.485	0.460	0.501	0.559	0.460	0.441	0.487	0.559	0.527	0.505	0.530	0.506
	VBN 1	0.504	0.446	0.380	0.443	0.504	0.490	0.456	0.483	0.504	0.486	0.453	0.481	0.469
	Mean	0.544	0.466	0.420	0.472	0.544	0.475	0.449	0.485	0.544	0.507	0.479	0.506	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
	(P=0.05)	0.014	0.018	0.018		0.026	0.032	0.032		0.045				

Fig. 17. Effect of flood stress on Fertility coefficient ratio



(0.483) over control (0.544). At growth stages, pod filling recorded higher fertility coefficient ratio (0.506) followed by flowering (0.485) and vegetative (0.472) stage. Interaction between variety and treatment indicated that CO 4 recorded higher reduction of fertility coefficient (19.2%) compared to VBN 1 (14.7%) by 10 days flooding. Interaction between treatment and stage showed that 10 days flooding recorded lesser ratio of fertility coefficient (0.423) compared to 5 days flooding (0.466) over control (0.344) at vegetative stage. Other interactions were significant.

4.2.13.4. Number of seeds per pod (Table 41)

Cowpea

There was significant difference among the varieties, treatments and stages. The mean values of number of seeds per pod were higher in CO 4 (13.2) compared to CO 2 (11.6). Irrespective of the varieties, flooding treatments differed significantly. Among the treatments, flooding for 10 days recorded lesser number of seed (10.6) compared to 5 days flooding (11.9) over control (14.8). At different stages, vegetative stage recorded higher number of seeds followed by flowering and pod filling stages. Interaction between varieties and treatments showed CO 2 recorded higher reduction of number of seeds per pod (35.6%) compared to CO 1 (21%) by 10 days flooding. Interaction between treatments and stage recorded that flooding for 10 days had lesser number of seeds per pod (9.1) followed by 5 days flooding (10.3) and control (14.8) at pod filling stage. Other interaction effect was non significant.

Soybean

Comparatively higher mean number of seeds per pod was recorded in the variety CO 1 (3.7) than in CO 2 (2.8). The varieties and treatments were significantly differed in their number of seeds per plant. Among the flooding treatments flooding for longer duration (10 days) recorded lesser number of seeds per pod (2.9) than shorter duration of flooding (5 days) (3.0) over control (3.9). The growth stages were non significant. The variety CO 2 recorded higher reduction in number of pods (30%) compared to CO 1 (22.4%) by 10 days flooding only. The interaction effect was non significant.

Table 41. Effect of flood stress on number of seeds per pod in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean				
	Control	Flooding for		Control	Flooding for		Control	Flooding for						
		5 days	10 days		Mean	5 days		10 days	Mean		5 days	10 days	Mean	
Cowpea	CO 4	15.0	14.4	13.0	14.1	15.0	13.1	12.9	13.4	15.0	10.9	10.3	12.0	13.2
	CO 2	14.6	12.5	11.4	12.8	14.6	10.6	9.0	11.4	14.6	9.6	7.9	10.7	11.6
	Mean	14.8	13.5	12.2	13.5	14.8	11.9	10.5	12.4	14.8	10.3	9.1	11.4	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.435	0.533	0.533		0.755	0.924	0.924		1.307				
Soybean	CO 1	4.4	3.6	3.4	3.8	4.4	3.4	3.5	3.8	4.4	3.3	3.3	3.7	3.7
	CO 2	3.4	2.8	2.2	2.8	3.4	2.5	2.6	2.8	3.4	2.5	2.3	2.7	2.8
	Mean	3.9	3.2	2.8	3.3	3.9	3.0	3.1	3.3	3.9	2.9	2.8	3.2	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.162	0.198	0.198		0.281	0.344	0.344		0.487				
Green gram	CO 4	7.0	6.5	6.4	6.7	7.0	5.6	5.1	5.9	7.0	4.5	4.4	5.3	6.0
	VBN 1	6.6	5.5	5.3	5.9	6.6	5.5	4.8	5.6	6.6	5.0	4.4	5.3	5.6
	Mean	6.8	6.0	5.9	6.2	6.8	5.5	5.0	5.8	6.8	4.7	4.4	5.3	
CD		V	T	S		VXT	TXS	VXS		VXTXS				
		0.426	0.522	0.522		0.739	0.905	0.739		1.280				

Green gram

Significant differences were noticed in both treatments and stages. No significant differences between the varieties. Among the varieties, CO 4 produced greater number of seeds per pod compared to VBN 1. The percentage of increase was 6.2. Among the treatments, flooding for 10 days reduced the number of seeds per pod (5.1) compared to 5 days flooding (5.4) over control (6.8). Irrespective of the treatments, the pod filling showed lower number of seeds per pod was 5.3 compared to flowering (5.8) and vegetative stage (6.2). Interaction effects were found to be nonsignificant.

4.2.13.5. 100 seeds weight (Table 42)

Cowpea

There was significant difference among the varieties. The overall mean of 100 seed weight was higher in CO 2 (10.95) than CO 4 (10.23). The treatments and stages had significant influences on the 100 seeds weight. Flooding treatments decreases the 100 seed weight. Among the flooding treatments flooding for 10 days recorded lower weight of 100 seeds (9.53) compared to 5 days flooding (9.81) over control (12.43). At different stages, pod filling recorded lower weight of 100 seed (9.95) followed by flowering (10.60) and vegetative (11.25) stages. Interaction between varieties and treatments showed the variety CO 2 recorded higher reduction in 100 seeds weight (28.3%) compared to CO 4 (17.7%) by 10 days flooding. Interaction between the treatments and stages revealed the flooding for 10 days observed maximum reduction in 100 seeds weight (31.1%) than 5 days flooding (28.8%) at pod filling stages. Other interactions were nonsignificant.

Soybean

The mean 100 seed weight was higher in CO 2 (11.84) than CO 1 (10.43). There was significant difference between the varieties, treatments and stages. Among the treatments, longer duration of flooding (10 days) decrease the 100 seed weight (9.76) compared to shorter duration of flooding (5 days) (10.21) over control (13.45). The effect of flooding for 5 days was not detrimental. Irrespective of the treatments,

Table 42. Effect of flood stress on 100 seeds weight (g) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean	
	Control	Flooding for		Control*	Flooding for		Control	Flooding for			
		5 days	10 days		Mean	5 days		10 days	Mean		5 days
Cowpea	CO 4	11.50	10.47	10.33	10.77	10.18	11.50	9.20	8.73	9.81	10.23
	CO 2	13.37	11.40	10.43	11.73	11.02	13.37	8.50	8.40	10.09	10.95
	Mean	12.43	10.93	10.38	11.25	10.60	12.43	8.85	8.57	9.95	
CD (P=0.05)	V	T	S		VXT	TX S	VXS	VXTXS			
	0.433	0.529	0.529	0.749	0.917	0.749	1.297				
Soybean	CO 1	12.63	10.57	10.33	11.18	10.18	12.63	8.77	8.73	10.04	10.43
	CO 2	14.27	12.27	11.37	12.63	12.17	14.27	9.20	8.73	10.73	11.84
	Mean	13.45	11.42	10.85	11.90	11.17	13.45	8.98	8.73	10.39	
CD (P=0.05)	V	T	S		VXT	TX S	VXS	VXTXS			
	0.484	0.592	0.592	0.838	1.026	0.838	1.452				
Green gram	CO 4	4.67	4.30	3.90	4.29	3.90	4.67	3.30	2.87	3.67	3.93
	VBN 1	4.30	3.37	3.37	3.78	3.39	4.30	2.70	2.57	2.19	3.45
	Mean	4.48	3.98	3.63	4.03	3.64	4.48	3.00	2.71	3.40	
CD (P=0.05)	V	T	S		VXT	TX S	VXS	VXTXS			
	0.356	0.436	0.436	0.617	0.756	0.617	1.069				

the growth stages differed significantly. The pod filling stages showed lesser weight of 100 seeds followed by flowering and vegetative stages. Interaction between treatments and stages showed that the longer duration of flooding produced lesser weight of 100 seeds (8.73) compared to shorter duration of flooding (8.98) over control (13.45) at pod filling stages. Other interactions were nonsignificant.

Green gram

Significant differences between the varieties, treatments and stages. The maximum 100 seed weight was observed in CO 4 (3.93) than other variety (VBN 1) (3.45). Flooding treatments decreases 100 seeds weight significantly. The variety CO 4 recorded higher seed weight than VBN 1 over all treatments at all stages. Among the treatments flooding for 10 days recorded higher reduction of 100 seeds weight (29.7%) than 5 days flooding (23.2%) over control. Irrespective of treatments, the stages differed significantly whereas the pod filling showed decrease in 100 seeds weight (3.40) followed by flowering (3.64) and vegetative stage (4.03). The interaction effects were nonsignificant.

4.2.13.6. Seed yield per plant (g^{-1}) (Table 43; Fig. 18)

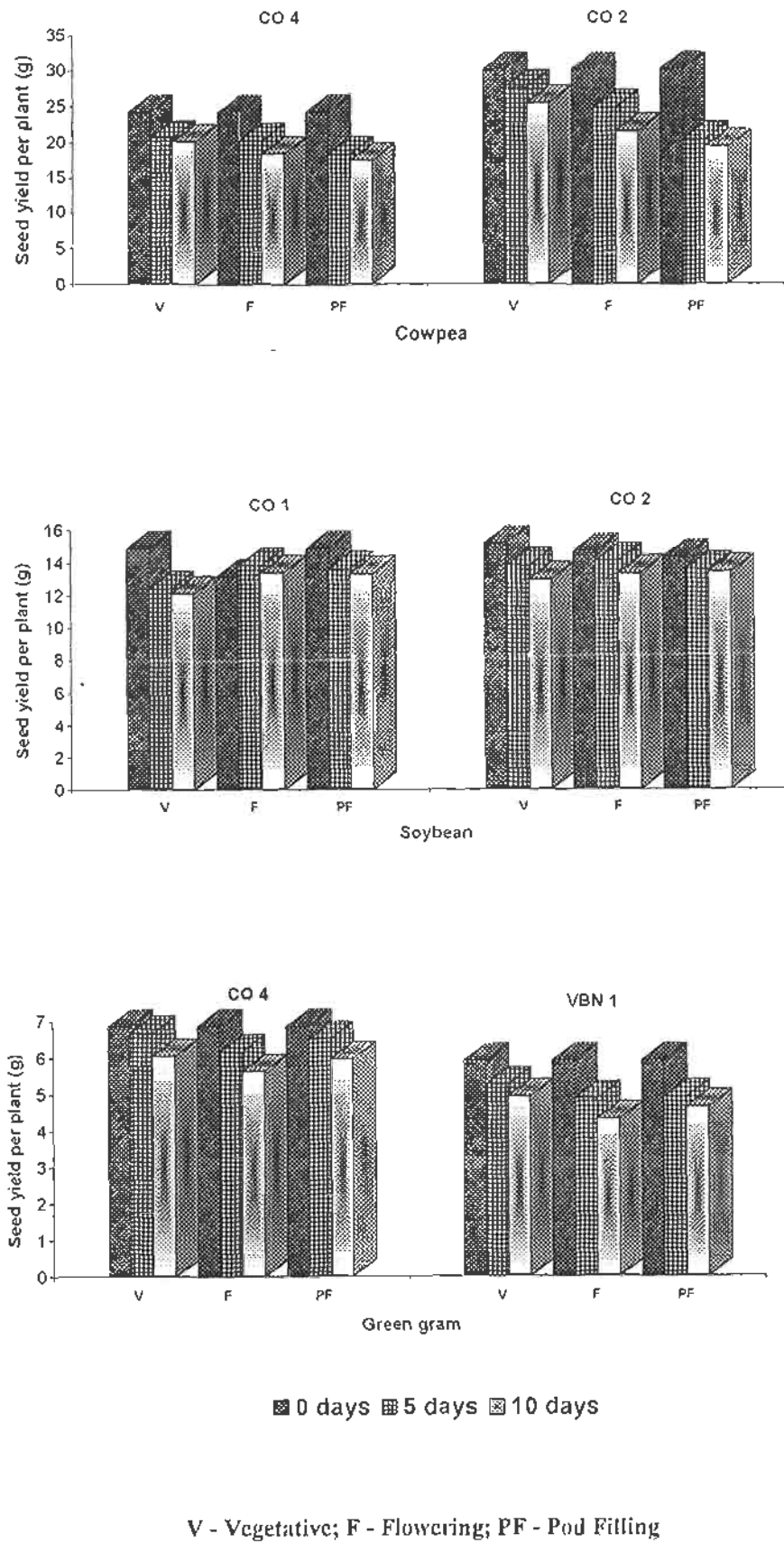
Cowpea

The varieties differed significantly in their productivity. The overall mean seed yield per plant was higher in CO 2 (25.45) than CO 4 (20.81). The difference among the varieties were found to be significant for flooding treatments. Among the treatments flooding for 10 days decreases seed yield per plant (20.32) drastically compared to 5 days flooding (21.95) over control (27.14). Irrespective of the treatments, vegetative stage recorded higher yield of seed per plant than flowering and pod filling stages. Interaction between varieties and treatments showed that CO 4 observed higher reduction in seed yield (26.90) by 10 days flooding compared to CO 2 (22.7%). Interaction between treatments and stages revealed that flooding for 10 days recorded lower seed yield per plant compared to 5 days flooding over control at pod filling stage. Interaction between varieties and stages indicated that CO 2

Table 43. Effect of flood stress on seed yield per plant (g) in pulses at different growth stages

Pulses	Vegetative stage			Flowering stage			Pod filling stage			Genotypes Mean			
	Control	Flooding for 5 days	Flooding for 10 days	Control	Flooding for 5 days	Flooding for 10 days	Control	Flooding for 5 days	Flooding for 10 days		Mean		
Cowpea													
CO 4	24.15	20.59	20.01	21.58	24.15	20.16	18.34	20.88	24.15	18.32	17.46	19.98	20.81
CO 2	30.13	27.54	25.36	27.67	30.13	24.73	21.45	25.44	30.13	20.33	19.28	23.25	25.45
Mean	27.14	24.07	22.69	24.63	27.14	22.45	19.89	23.16	27.14	19.33	18.37	21.61	
CD (P=0.05)	V	T	S	VXT	VXT	TXS	VXS	VXS	VXTXS	VXTXS	VXTXS	VXTXS	
	0.602	0.737	0.737	1.043	1.043	1.277	1.043	1.043	1.806				
Soybean													
CO 1	14.84	12.42	12.13	14.46	13.13	13.84	13.40	14.03	14.84	13.66	13.34	13.95	14.15
CO 2	15.17	13.83	12.97	13.99	14.68	14.11	13.34	14.04	14.34	13.56	13.44	13.78	13.94
Mean	15.01	12.12	12.55	14.22	13.91	13.98	13.37	14.04	14.59	13.61	13.39	13.87	
CD (P=0.05)	V	T	S	VXT	VXT	TXS	VXS	VXS	VXTXS	VXTXS	VXTXS	VXTXS	
	0.448	0.549	0.549	0.776	0.776	0.950	0.776	0.776	1.344				
Green gram													
CO 4	6.85	6.67	6.06	6.53	6.85	6.17	5.64	6.22	6.85	6.52	5.97	6.45	6.40
VBN 1	5.91	5.26	4.93	5.37	5.91	4.87	4.32	5.03	5.91	4.92	4.65	5.16	5.19
Mean	6.38	8.97	5.50	5.95	6.38	5.52	4.98	5.63	6.38	5.72	5.31	5.80	
CD (P=0.05)	V	T	S	VXT	VXT	TXS	VXS	VXS	VXTXS	VXTXS	VXTXS	VXTXS	
	0.396	0.485	0.485	0.686	0.686	0.840	0.686	0.686	1.189				

Fig. 18. Effect of flood stress on Seed yield per plant (g)



172

recorded higher yield of seed per plant (27.67) as compared to CO 4 (21.58) at vegetative stage.

Soybean

Flooding significantly reduced the seed yield per plant. Severe reduction in seed yield was caused by 10 days flooding. There is no significant difference between the varieties in their seed yield. Flooding for 10 days showed reduction in seed yield (9.1%) compared to 5 days flooding (6%). At different growth stages, the pod filling was the most sensitive stage followed by flowering and vegetative stages. Interaction effects were non-significant.

Green gram

Significant variations between the varieties and treatments for their seed yield per plant. The mean seed yield per plant was maximum in CO 4 (6.40) compared to VBN 1 (5.19). Flooding reduces the seed yield per plant significantly. Among the treatments flooding for 10 days decreases the seed yield by 17.6 per cent as compared to 5 days flooding (10.2%) over control. No significant differences between the stages. Interaction effects were non significant.

4.2.14. Molecular Mechanism

4.2.14.1. Study of leaf and root protein profiles in pulses under flooded condition

(Table 44) (Plate 6)

The SDS - PAGE profile of protein extracts from the cowpea (CO 4 and CO 2), soybean (CO 1 and CO 2) and green gram (CO 4 and VBN 1). Under control and flooded for 5 and 10 days flooding conditions revealed that, there was detectable difference between control and flooded condition in leaves and roots. Though many slight variations were observed in protein profiles for leaves and roots. Prominent detectable variation was also taken into consideration. The molecular weight of bands is approximated based on Rm. values. The observed variation is given in the table 45 and the electrogram is presented in plate.

From the table it was evident that there was detectable differences between flooded and control samples of pulses.

Cowpea

Anaerobic proteins synthesized in roots under flooded conditions. Under 5 and 10 days of flooding the variety CO 2 showed a prominent expression of 98, 83 and 43 kDa protein in leaves compared to CO 4 variety over control. In roots, the molecular weight proteins of 23, 43 and 75 kDa expressed well in CO 4 variety during 5 and 10 days of flooding. However the variety CO 2 had no expression of 43 kDa anaerobic proteins in roots under flooded treatments.

Soybean

There were little qualitative and quantitative differences in protein profiles between the varieties. The variety CO 2 had reduction in the expression of higher molecular weight protein (88 and 98 kDa) and also a lower molecular weight protein (14 kDa) in leaves under 5 and 10 days of flooding. However, the variety CO 1 showed higher expression of 43 and 98 kDa protein in leaves under flooded conditions. In roots, the variety CO 1 marked the higher enhancement in 18, 23 and 43 kDa proteins during 5 and 10 days flooding, but lower intensity in CO 2 variety.

Green gram

The anaerobic polypeptides were expressed poorly in green gram varieties. Both varieties (CO 4 and VBN 1) had lesser expression of 14, 88 and 98 kDa proteins in leaves under flooded conditions over control. However, the Rubisco protein (43 kDa) expressed well in both the varieties (CO 4 and VBN 1). Regarding anaerobic root proteins a lower molecular weight proteins (23 kDa) expressed well in both varieties during 5 and 10 days flooding compared to control. But the 43 kDa protein expressed well in CO 4 compared VBN 1 under 5 day and 10 day flooded situations. The other proteins showed a poor expression or absent in flooded situations.

Table 44. Study of SDS-PAGE leaf and root protein profiles in pulses under flooded conditions

Pulses	Molecular weight (kDa)	Leaf		Molecular weight (kDa)	Root			
		Control	Flooding for		Control	Flooding for		
			5 days			10 days	5 days	10 days
Cow pea	14	++	+	23	*	*		
	43	**	**	43	*	-		
	83	*	+	75	*	*		
	98	-	+	-				
	14	++	+	23	++	++		
	43	**	**	43	**	**		
Soybean	83	++	+	75	*	*		
	98	-	*	-				
	14	++	++	18	**	*		
	43	**	**	23	**	*		
	88	**	+	43	*	*		
	98	+	+	75	-	+		
Green gram	14	+	+	18	+	++		
	43	**	**	23	+	++		
	88	+	+	43	+	++		
	98	*	**	75	-	-		
	14	++	+	23	+	++		
	43	**	**	43	+	+		
VBNI	88	*	*	75	*	-		
	98	*	+	-				
	14	++	+	23	*	**		
	43	**	*	43	+	-		
	88	++	+	75	+	-		
	98	++	+	-				

Legend : ++ High intensity + Low intensity ** Thick band * Thin band - Absent

4.2.14.2. Isozyme analysis

Peroxidase (Plate 7)

Cowpea

The isozyme pattern of peroxidase revealed the presence of different isoforms in the leaves and roots of CO 4 and CO 2. Difference in number of bands between flooded for 5 and 10 days and control were observed in the plate. Under control, six bands were observed in leaves of the varieties CO 4 and CO 2. At 5 days flooding, the variety CO 4 produced six bands compared to CO 2 which expressed 4 bands. But at 10 days flooding the number of bands reduced in CO 2 by 3 and in CO 4 by 5. However, the intensity and thickness of the bands are also less under flooded treatments. In roots under control condition both the varieties CO 4 and CO 2 produced 6 bands each respectively, where as under flooding for 5 days and 10 days produced 6 and 5 bands in CO 4 and 5 bands in CO 2. However the intensity of bands are also more under flooded conditions.

Soybean

Isoenzymatic pattern of peroxidase enzyme is depicted in plate 7. In this banding sequence, CO 1 and CO 2 produced 5 and 4 bands in leaves under control where as flooding treatments like 5 days and 10 days flooding produced 6 and 6 bands in CO 1 and 6 and 5 bands in CO 2 variety. The intensity of isoforms were high only in CO 1 under flooded conditions. In control, both the varieties CO 1 and CO 2 produced 9 bands each in control. The variety CO 2 produced 8 isoforms each under 5 and 10 days of flooding namely., 2,3,4,5,6,7,8 and 9 over control. In CO 1 produced 10 numbers of isoforms over control. However the intensity of the bands were less under flooded conditions.

Green gram

The distribution of peroxidase isoforms were presented in Plate 7. Irrespective of the genotypes, flooded for 5 days had 5 isoforms where as 10 days flooding had 3 isoforms over control (5 isoforms). The leaves of VBN 1 produced 5 number of isoforms compared to CO 4 (4 isoforms). In roots, the variety CO 4 produced 6 isoforms compared to VBN 1 it had 5 isoforms. Under flooded condition. 10 days

flooding decrease the expression of isoforms as 3 in VBN 1 and 4 in CO 4 compared to 5 days flooding produces 4 isoforms in VBNI and 5 isoforms in CO 4.

4.2.15. Morphological and anatomical changes associated with flooding tolerance in pulses

4.2.15.1. Adventitious roots and stem cracking (Plate 3)

Roots and shoots of flood-tolerant crops show distinctive changes in their morphology and anatomy during flooding that can be viewed either as symptoms of flooding injury or as acclimatize responses. Present study showed that flooded pulses exhibited pronounced lower stem hypertrophy (swelling of cells), adventitious roots and aerenchyma formation. After 5 days of flooding, pulses like cowpea, Soybean and green gram produced several long adventitious roots that only emerged from the stem at or just above the root collar. The stem hypertrophied revealed that stem enlargement in pulses only occur in cortical layer, which is the site where adventitious roots and aerenchyma developed. After flooded pots were allowed to drain, the adventitious roots darkened and began growing downward in the soil. The degradation of old roots and the development of adventitious roots under flooded conditions can result in shallower rooting depths.

In cowpea, the variety CO 4 produced higher amount of adventitious roots than CO 2. Stem cracking or stem hypertrophy was also present in both varieties. Adventitious roots were completely absent in non-flooded varieties.

In soybean, there was no differentiation between the varieties (CO 1 and CO 2) for production of adventitious roots and also exhibits cracking of stem or swelling of the stem base. Irrespective of the varieties, the adventitious roots were highly clustered together above the soil layer and also presence of accelerated cracking or swelling of the stem.

In green gram, the 5 days flooding showed that many old roots of both the variety (CO 4 and VBN 1) dies under flooding treatment by numerous adventitious roots developed. However the variety CO 4 produced more adventitious roots than VBN 1 and clustered together above the water surface. In non flooded varieties had no adventitious roots and swelling of stem.

4.2.15.2. Aerenchyma development (Plate 5)

The microtomy section of flooded and control roots were performed to study the aerenchyma development. The paraffin wax sections were examined carefully under microscope. It was observed that in flooded pulses roots had aerenchyma formation. Flooding resulted in aerenchyma proliferation in the cortex of roots, especially in adventitious roots. The formation of aerenchyma in flooded pulse roots were normally accomplished by an extensive lysis of cortical cells through cell disintegration (lysigenous). Aerenchyma tissue with large intercellular spaces, giving continuous gas-filled channels from the shoots to the roots. The cortical cells of roots of nonflooded pulses were tightly packed and no aerenchyma was observed.

In cowpea, the variety CO 4 observed that extent of aerenchyma development was more (become well developed) in roots compared to CO 2 under flooding for 5 days. Irrespective of varieties, the aerenchyma development was external to the endodermis and peripheral to the cortex.

In soybean, the enhancement in aerenchyma formation was observed higher in both varieties (CO 1 and CO 2) by 5 days flooding. There was no formation of aerenchyma in control roots.

In green gram, the formation of aerenchyma was well developed in CO 4 compared to VBN 1 on 5 days flooding. The configuration of aerenchyma was mainly at center to the peripheral region of cortex. The lacunae or air filled spaces was more in CO 4 compared to VBN 1.

4.3. Chemical manipulation for induction of flooding tolerance in Green gram

Screening of chemicals and plant growth regulators for the induction of flooding tolerance and also to identify the correct stage of spraying for increasing the yield.

4.3.1. Yield components

4.3.1.1. Number of flowers per plant (Table 45; Fig. 19)

The number of flowers produced per plant were recorded at flowering and pod filling growth stages. Flooding stress significantly decreases the flower production. The overall mean of number of flowers per plant was highest in CO 4 (35.06) and lowest in VBN 1 (30.79). There was a significant variation between the varieties, treatments and stages. Among the treatments the treatment T₂ (Flooded control) recorded higher reduction in number of flower (29.7%) over control (T₁). However, the treatment T₅ and T₈ showed lesser reduction in flower number per plant (10.6% and 12.5%) compared to T₂ (13.8%) over control (T₁). Among the stages, pod filling recorded higher number of flowers per plant (34.18) than flowering stage (32.14). Interaction between treatments and stages showed that the treatment T₈ recorded lower reduction in number of flowers per plant (8.9%) compared to T₂ (28%) over control at pod filling stages. Interaction between the varieties and treatments and stages revealed that the CO 4 recorded higher number of flowers (40.17) on T₅ at pod filling stage. Other interaction effects were non significant.

4.3.1.2. Number of pods per cluster (Table 45)

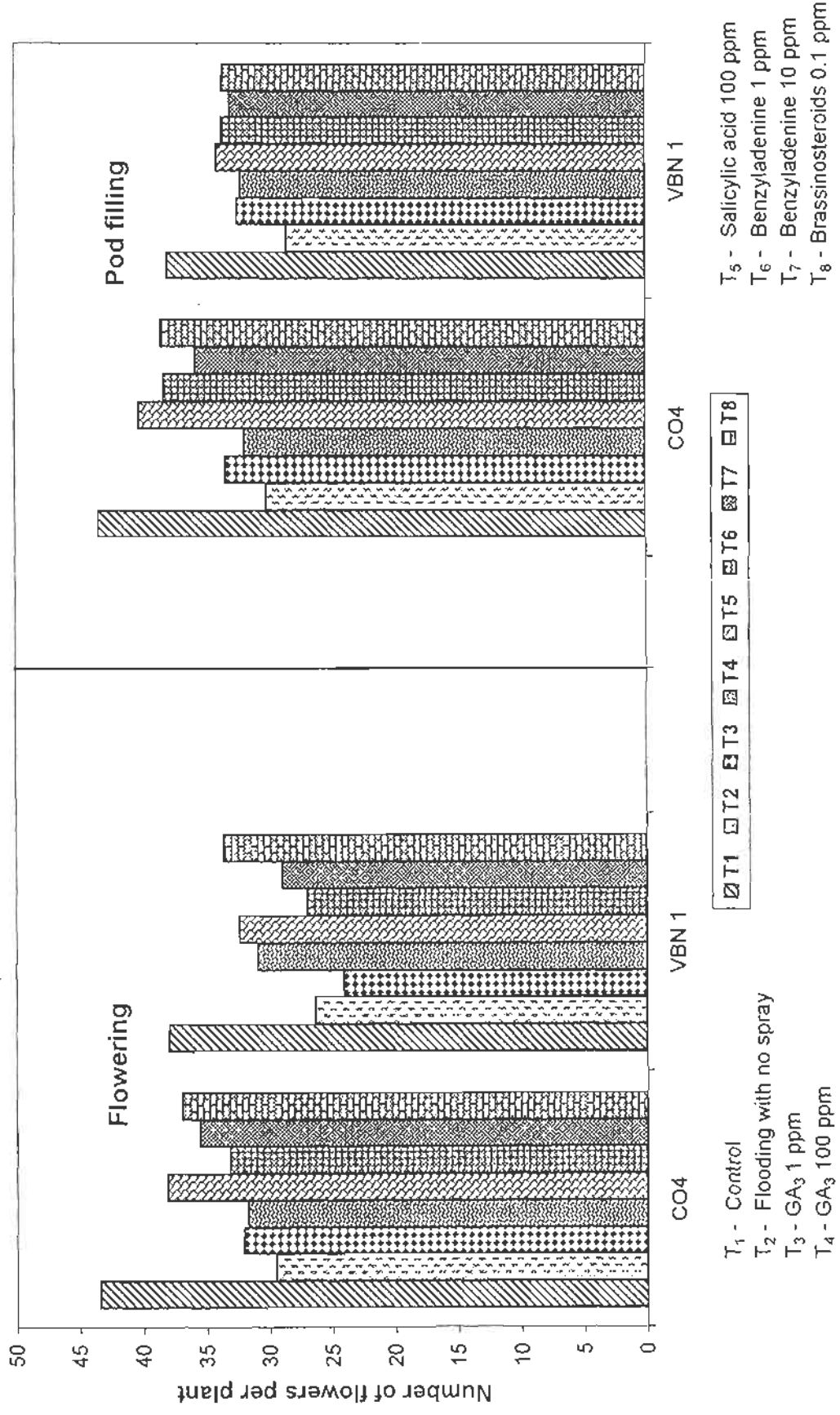
Number of pods per cluster was significantly decreased by the flooding stress. There is no significant variation between the varieties. Among the different biochemical sprayed, the treatment T₈, T₅ and T₄ recorded higher number of pods per cluster (3.29, 3.27 and 3.19) compared to T₂ (2.83). At growth stages, pod filling recorded higher number of pods per cluster (3.22) as compared to flowering (3.15) stages. However, the CO 4 recorded mean number of pods per cluster was 3.25 compared to VBN 1 (3.12). Interaction between the varieties and treatments showed

Table 45. Effect of ameliorants on number of flowers per plant and number of pods per cluster under 5 days flooding in green gram varieties

Treatments	Number of flowers per plant						Number of pods per cluster					
	Flowering			Pod filling			Flowering			Pod filling		
	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean
T1	43.45	37.94	40.71	43.45	37.94	40.71	3.57	3.40	3.48	3.57	3.40	3.48
T2	29.53	26.36	27.94	30.11	28.51	29.31	2.96	2.94	2.95	2.94	2.63	2.79
T3	32.07	24.10	30.59	33.35	32.41	29.91	3.03	3.14	3.08	3.21	3.13	3.17
T4	31.72	30.93	28.83	31.84	32.13	30.18	3.08	3.10	3.08	3.27	3.15	3.21
T5	38.11	32.33	35.72	40.17	33.98	37.07	3.34	3.19	3.26	3.34	3.28	3.31
T6	33.23	27.06	30.15	38.23	33.63	35.93	3.14	3.01	3.07	3.27	3.09	3.18
T7	35.56	28.96	32.26	35.74	32.98	34.36	3.14	3.06	3.10	3.39	3.15	3.27
T8	36.93	33.63	35.28	38.43	33.57	35.98	3.26	3.13	3.20	3.41	3.26	3.33
Mean	35.06	30.79	32.14	36.14	33.02	34.18	3.19	3.12	3.15	3.31	3.12	3.22

V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS
 0.387 0.774 0.387 1.095 1.095 1.095 1.549 0.055 0.111 0.055 0.156 0.078 0.222
 CD (P=0.005)

Fig. 19. Effect of ameliorants on number of flowers per plant in greengram



that CO 4 recorded higher number of pods per cluster on par at T₅ and T₈. Interaction between treatments and stages indicated that the T₈ recorded lesser reduction in number of pods per cluster (4.3%) next followed by T₅ (4.9%) and T₇ (6.0%) compared to other treatment at pod filling stages.

4.3.1.3. Number of pods per plant (Table 46)

The biochemicals spray had a distinct effect on the number of pods per plant, which is a determining factor for final crop yield. Among the varieties CO 4 recorded higher number of pods than VBN 1. Irrespective of the varieties the treatment T₈ (20.7) and T₅ (20.3) recorded higher number of pods per plant compared to T₂ (15) but lower to T₁ (24.8). No significant difference between the stages. Interaction between varieties and treatment showed that CO 4 recorded higher number of pods at T₅ and T₈ treatments. Interaction between varieties and stages revealed that the CO 4 had higher number of pods compared to VBN 1 at pod filling stages.

4.3.1.4. Fertility coefficient (Table 46; Fig. 20)

The varieties showed significant difference for fertility coefficient. The variety VBN 1 recorded highest ratio of fertility coefficient (0.570) than lowest in CO 4 (0.541). Among the different treatments, T₈ recorded highest ratio of fertility coefficient followed by T₇, T₄, T₃ and compared to T₂. At two stages, flowering shows higher fertility coefficient ratio (0.566) compared to pod filling stage (0.545). Interaction between varieties and treatments showed that CO 4 recorded higher fertility coefficient ratio (0.583) at T₄ and VBN 1 (0.589) at T₈. Interaction between treatments and stages indicated that T₄ recorded higher fertility coefficient followed by T₈, T₃ and T₇ at pod filling stages.

4.3.1.5. Number of seeds per pod (Table 47)

Comparatively higher number of seeds per pod was recorded in the variety CO 4 (5.2) than VBN 1 (4.7). Among the treatments the T₅ and T₈, T₃ and T₄, T₆ and T₇ on par with each other for number of seeds per pod and higher than T₂ and lower than T₁. No significant difference between the stages. Interaction between treatments and

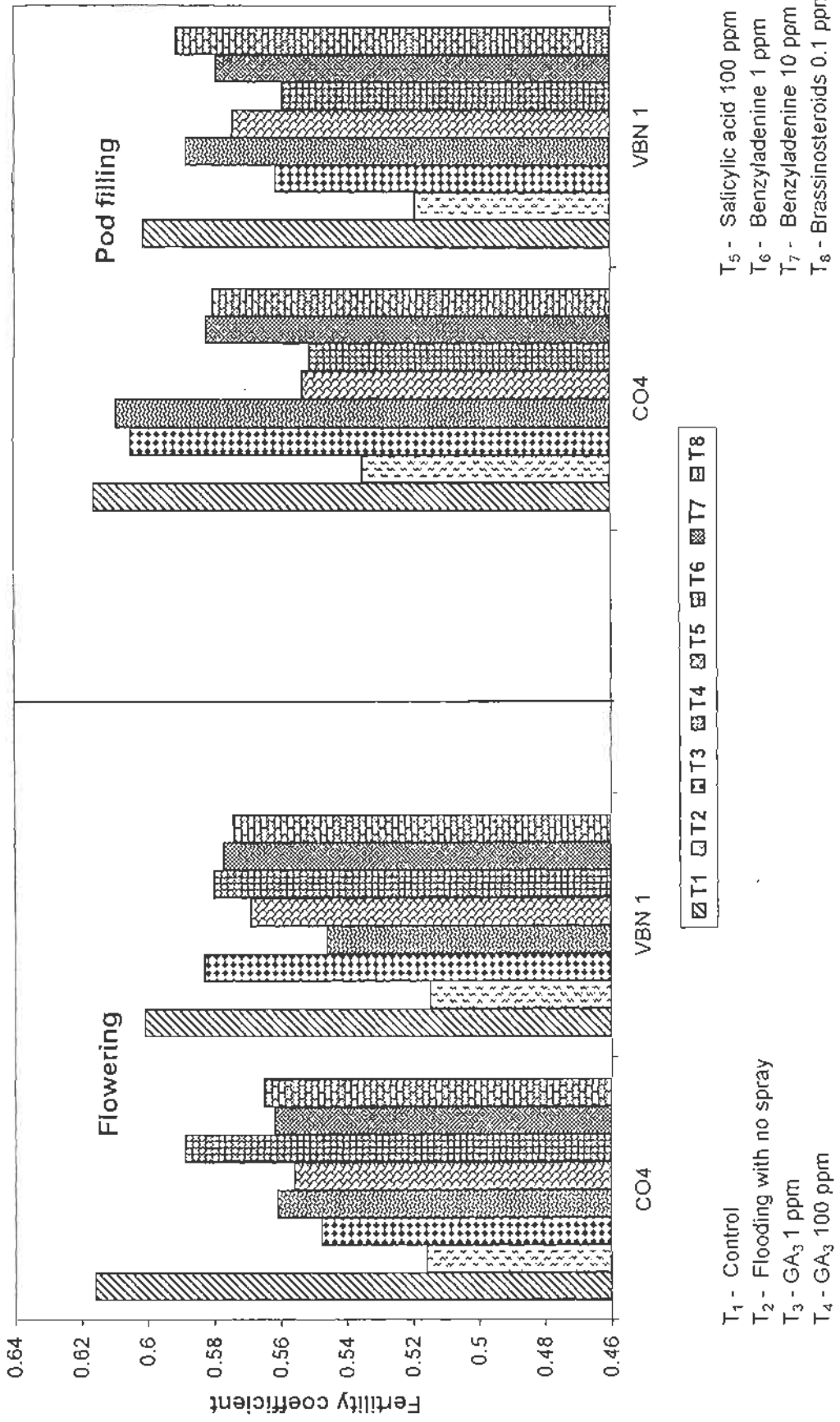
Table 46. Effect of ameliorants on number of pods per plant and fertility coefficient under 5 days flooding in green gram varieties

Treatments	Number of pods per plant						Fertility coefficient					
	Flowering			Pod filling			Flowering			Pod filling		
	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean
T1	26.8	22.8	24.8	26.8	22.8	24.8	0.616	0.601	0.609	0.616	0.601	0.609
T2	15.4	13.6	14.5	16.1	14.8	15.5	0.516	0.515	0.516	0.535	0.519	0.527
T3	19.0	16.8	17.9	20.1	18.1	19.9	0.548	0.583	0.566	0.605	0.561	0.583
T4	18.7	16.9	17.8	19.8	18.3	19.3	0.561	0.546	0.554	0.609	0.588	0.599
T5	20.9	18.4	19.7	22.1	19.6	20.9	0.556	0.569	0.563	0.553	0.574	0.563
T6	19.5	15.7	17.6	21.3	18.8	20.1	0.589	0.580	0.585	0.551	0.559	0.555
T7	20.0	16.7	18.4	21.4	19.1	20.3	0.562	0.577	0.570	0.582	0.579	0.581
T8	20.9	19.3	20.1	22.4	19.8	21.1	0.565	0.574	0.570	0.580	0.591	0.586
Mean	20.5	18.5	19.5	21.3	18.9	20.1	0.564	0.568	0.566	0.517	0.572	0.545

V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS

CD 0.350 0.701 0.350 0.991 0.991 0.991 1.402 0.013 0.027 0.013 0.038 0.038 0.019 0.054
(P=0.005)

Fig. 20. Effect of ameliorants on fertility coefficient in greengram



stages revealed that T₈ produced higher number of seeds per pod at flowering stage and T₁ at pod filling stage. Interaction between varieties and stages showed that CO 4 produced more number of seeds per pod (5.2) compared to VBN 1 (4.6) at flowering.

4.3.1.6. Hundred seed weight (g) (Table 47)

There was significant difference among the varieties. The maximum 100 seed weight was with CO 4 (4.0) than the other variety VBN 1 (3.2). Irrespective of the varieties, the treatments differed significantly. The T₁ recorded greater seed weight (4.3) compared to T₈ (2.9) followed by T₅ (3.8), T₆ and T₇ (3.6). Among the stages flowering recorded higher seed weight (3.7) compared to pod filling (3.4). Interaction between varieties and stages indicated that CO 4 and VBN 1 recorded higher seed weight (4.2 and 3.3) at flowering stages.

4.3.1.7. Seed yield per plant (Table 48)

Significant differences between the varieties for seed yield per plant. The variety CO 4 recorded higher mean seed yield per plant (5.90) than VBN 1 (4.91). Irrespective of the varieties the treatment T₈ and T₅, T₆ and T₇ were at par with each other for seed yield per plant. However, the T₈ and T₅ recorded maximum seed yield per plant (5.56) compared to T₂ (4.93) but lower to T₁ (6.79). However, the T₈ and T₉ showed that percentage of decrease was 12.2% compared to unsprayed T₁ (19.8%). At different stages, the flowering recorded higher seed yield per plant (5.57) than pod filling stage (5.23). Interaction between varieties and treatments showed that CO 4 (5.98 and 5.94) recorded higher seed yield per plant than VBN 1 (5.13 and 5.18) at T₅ and T₈. The variety CO 4 showed lesser reduction in seed yield per plant (3.7%) under T₅ compared to T₂ (18.7%) at flowering stage.

4.3.1.8. Biological yield (kg ha⁻¹) (Table 48)

There was significant difference when the varieties were compared for biological yield. The variety CO 4 recorded maximum biological yield (2482 kg ha⁻¹) where as VBN 1 recorded only 1964 kg ha⁻¹. Among the different treatments, T₅ recorded higher biological yield (2400) followed by T₈ (2355) and T₆ (2240)

Table 47. Effect of ameliorants on number of seeds per pod and hundred seeds weight (g) under 5 days flooding in green gram varieties

Treatments	Number of seeds per pod										Hundred seeds weight (g)					
	Flowering			Pod filling			Flowering			Pod filling						
	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean				
T1	5.7	5.3	5.5	5.7	5.3	5.5	4.8	3.8	4.3	4.8	3.8	4.3				
T2	4.5	3.9	4.2	4.0	4.0	4.0	3.5	2.7	3.1	3.0	2.5	2.7				
T3	5.1	4.1	4.6	5.0	4.3	4.6	4.0	3.0	3.5	3.4	2.7	3.1				
T4	5.1	4.6	4.5	5.0	4.4	4.7	3.9	3.1	3.5	3.3	2.8	3.1				
T5	5.3	4.9	5.1	5.5	5.0	5.2	4.5	3.5	4.0	3.9	3.2	3.6				
3.6	5.4	4.5	4.9	5.3	4.8	5.1	4.0	3.4	3.7	3.7	3.3	3.5				
T7	5.5	4.5	5.0	5.2	4.8	5.0	4.2	3.3	3.7	3.7	3.0	3.4				
T8	5.7	4.9	5.3	5.2	5.1	5.1	4.6	3.6	4.1	4.0	3.4	3.7				
Mean	5.3	4.6	4.9	5.1	4.7	4.9	4.2	3.3	3.7	3.7	3.0	3.4				

V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS

CD 0.171 0.343 0.171 0.485 0.485 0.242 0.686 0.113 0.226 0.113 0.320 0.320 0.160 0.452
(P=0.005)

Table 48. Effect of ameliorants on seed yield per plant (g) and biological yield (kg/ha) under 5 days flooding in green gram varieties

Treatments	Seed yield per plant						Biological yield per hectare (kg/ha)					
	Flowering			Pod filling			Flowering			Pod filling		
	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean	CO4	VBN 1	Mean
T1	6.79	5.51	6.15	6.79	5.51	6.15	2754	2135	2445	2757	2135	2445
T2	5.52	4.39	4.96	5.32	4.48	4.90	2087	1648	1868	2099	1750	1907
T3	6.02	4.73	5.38	5.17	4.48	4.82	2196	1867	20.38	2373	1985	2179
T4	6.10	4.59	5.35	5.25	4.52	4.89	2229	1855	2042	2371	2050	2211
T5	6.54	5.03	5.79	5.41	5.23	5.32	2729	2004	2367	2723	2100	2412
T6	6.23	4.91	5.57	5.42	5.03	5.22	2354	1891	2123	2649	2064	2357
T7	6.31	4.92	5.62	5.51	4.83	5.17	2376	1878	2077	2491	2057	2275
T8	6.38	5.16	5.77	5.49	5.20	5.34	2713	1916	2315	2704	2085	2395
Mean	6.24	4.91	5.57	5.55	4.91	5.23	2430	1899	2157	2533	2028	2273

V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS
 CD 0.010 0.021 0.010 0.030 0.030 0.015 0.042 31.888 63.777 31.888 90.195 90.195 45.097 127.555
 (P=0.005)

compared to T₂ (1888). At growth stages, pod filling recorded higher biological yield than flowering stage. Interaction between varieties and treatments showed that CO 4 recorded higher biological yield (2726) compared to VBN 1 (2052) at T₅. Interaction between treatments and stages revealed that T₅ recorded higher biological yield (2412) than T₂ (1907) at pod filling stages

4.3.1.9. Seed yield (kg ha⁻¹) (Table 49; Fig. 21)

As like that of seed yield per plant, the significant difference between the varieties. The variety CO 4 (1247) showed higher seed yield per hectare than VBN 1 (695). Irrespective of the varieties, the T₈ and T₅ recorded lesser reduction in seed yield per hectare (3.2 and 3.5%) compared to T₂ (31%). Among the stages pod filling recorded higher seed yield per hectare (966) than flowering (955) stage. Interaction between varieties and treatment indicted that T₈ caused lower reduction of 0.2 % and 7.4 % in CO 4 and VBN 1 respectively. The interaction between treatments and stages revealed that pod filling showed higher seed yield than flowering stage at T₅ (1020 and 1008) and T₈ (1029 and 1014) over T₂ (734 and 710).

4.3.1.10. Harvest index (Table 49)

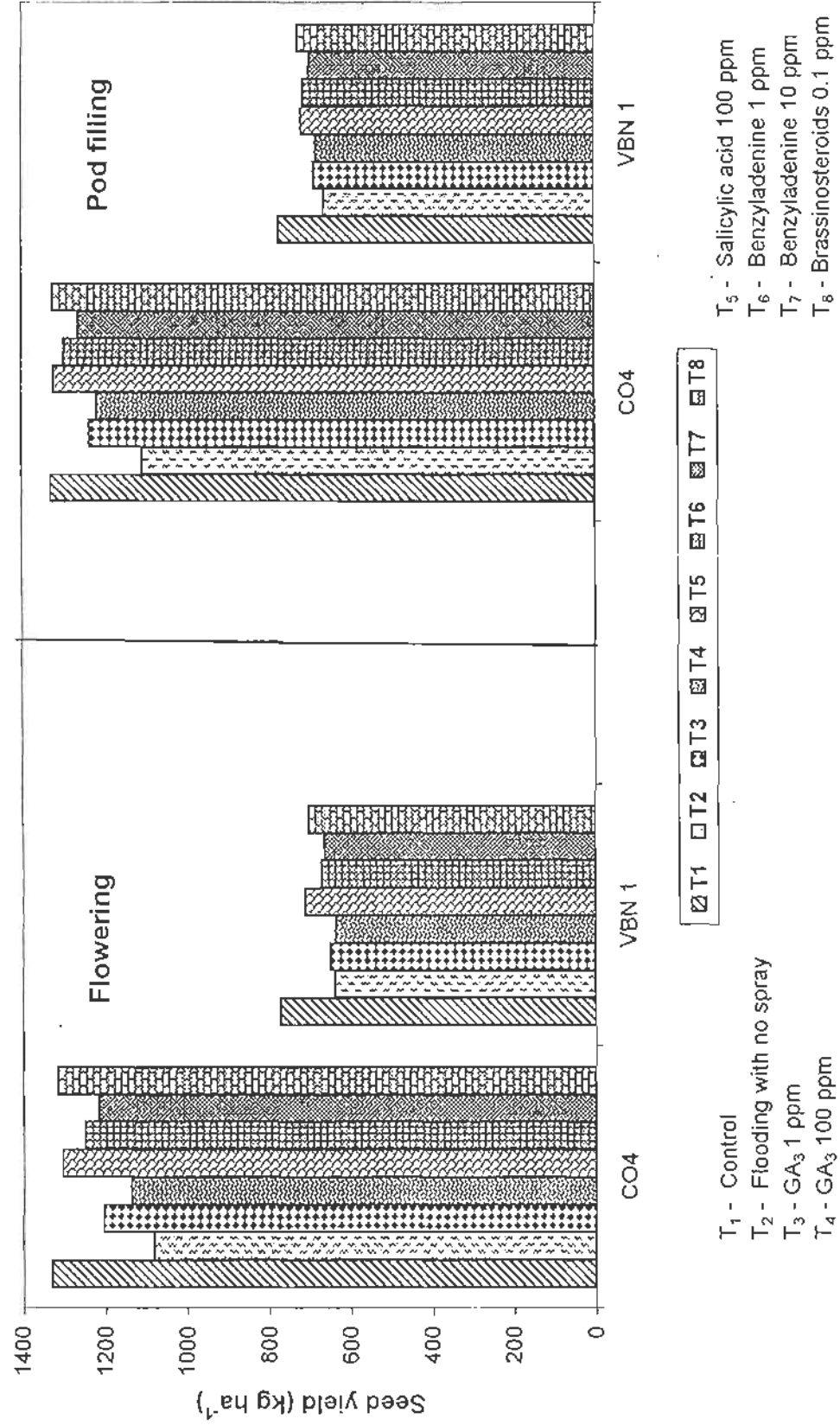
Irrespective the treatment the varieties differed significantly from each other. In which the variety CO 4 recorded higher harvest index (0.49) than VBN 1 (0.35). Among the treatments T₅ and T₈ recorded highest harvest index value (0.42) than T₂ (0.32). Irrespective of the treatments, there was less significant difference between the stages. Interaction between varieties and treatments showed that T₈ caused higher harvest index in CO 4 and T₆, T₈ in VBN 1. Interaction between treatments and stage revealed that T₃ followed by T₄, T₅, T₆, T₇ and T₈ recorded higher harvest index at pod filling stage.

Table 49. Effect of ameliorants on seed yield (kg/ha) and harvest Index under 5 days flooding in green gram varieties

Treatments	Seed yield (kg/ha)						Harvest Index					
	Flowering			Pod filling			Flowering			Pod filling		
	CO4	VBN I	Mean	CO4	VBN I	Mean	CO4	VBN I	Mean	CO4	VBN I	Mean
T1	1330	773	1051	1330	773	1051	0.48	0.36	0.43	0.48	0.36	0.43
T2	881	539	710	903	564	734	0.42	0.33	0.38	0.43	0.32	0.38
T3	1205	650	927	1235	688	962	0.54	0.35	0.45	0.52	0.35	0.44
T4	1137	637	887	1217	683	950	0.51	0.34	0.43	0.51	0.33	0.42
T5	1305	712	1008	1321	718	1020	0.48	0.36	0.42	0.49	0.34	0.42
T6	1250	672	961	1297	713	1005	0.53	0.36	0.45	0.49	0.35	0.42
T7	1215	664	939	1261	698	980	0.51	0.36	0.44	0.51	0.34	0.42
T8	1315	703	1009	1324	728	1026	0.48	0.37	0.42	0.49	0.35	0.42
Mean	1205	669	955	1236	696	966	0.49	0.35	0.43	0.49	0.34	0.42

V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS V T S VXT TXS VXT VXTXS
 CD 2.799 5.599 2.799 7.918 7.918 7.918 3.959 11.198 0.005 0.011 0.005 0.016 0.016 0.008 0.231
 (P=0.005)

Fig. 21. Effect of ameliorants on seed yield (kg ha⁻¹) in greengram



Discussion

CHAPTER V

DISCUSSION

Flooding of soils had a major impact on agriculture throughout the history. The adverse consequences of flooding are, of course, due to the damage it causes to all. No systematic research has been done on the relative tolerance of crop plants to waterlogging, but it is recognized that leguminous crops such as peas and beans are particularly sensitive. To take one example from a tropical environment, waterlogging is regarded as a major problem on the growth of pigeonpea crop raised in deep vertisols in India (Reddy and Virmani, 1981)). Crop species vary widely in their response to flooded conditions, and legumes with a few exceptions, are generally regarded as most sensitive species.

Under waterlogged condition, the anaerobic environment of the root zone affects plant metabolism as well as nutrient and water uptake by roots. Thus, productivity of most agricultural crops is lowered. Efforts to improve the productivity of pulses under water excess condition needs understanding on the mechanism, so as to identify traits required for productivity improvement programmes. Flooding resistance is the manifestation of morpho-physiological, biochemical and molecular mechanisms, which allow the crop to survive, grow and maximize yield under water excess conditions. As there is only limited works on screening pulses for flooding tolerance, emphasis must be given for this area of research.

In the present study, an attempt has been made to elucidate information on flooding tolerance in pulses with an effort to identify flood tolerant traits and means to mitigate the ill effect of flooding through chemical ameliorants. The results obtained are discussed in this chapter with limited support from earlier studies.

5.1. Screening of pulses for flooding tolerance under laboratory condition

5.1.1. Triphenyl Tetrazolium Chloride (TTC) reduction method

Reduction of 2-3-5 triphenyl tetrazolium chloride, known as the TTC method, indicate the status of oxidation-reduction reactions in the cell (Palta *et al.*, 1981). The measure of TTC reduction ability can thus be taken as indicative of the rate of respiration in the tissue. It is reported that during flooding of plants, the tolerance to waterlogging will be directly related to the extent of TTC reduction ability maintained by the tissue. In the present study, among the different pulses tested for flooding tolerance, the green gram variety, CO 4 had higher amount of TTC reduction ability than other varieties. Increasing ability to reduce TTC indicates increasing flood tolerance. Also tolerant varieties had capability to carry out electron transport. In cowpea, the variety CO 4 showed higher TTC reducing ability than CO 2. However, in other pulses like red gram, bengal gram and black gram there were no significant differences between the varieties for TTC reducing ability. But in soybean the varieties like CO 1 and CO 2 had less significant differences for root respiration. This is in accordance with the findings of Nelson *et al.* (1983) in beans.

5.1.2. Electrical Conductivity (EC) Test

A conductivity method (Dexter *et al.*, 1932) measuring ion efflux following stress injury has been used successfully to evaluate relative freezing and heat-stress tolerance of potato species (Palta *et al.*, 1981). In general, the response trend of the genotypes to flooding was similar to that of the TTC experiment, the CO 4 tolerant and VBN 1 susceptible in green gram and in cowpea the CO 2 susceptible and CO 4 tolerant with a significant differences between ion solutes leakage. The other pulses had low variability (no differences) in ion leakage from root segments. The above said observation is in accordance with Nelson *et al.* (1983). Lower membrane stability due to higher injury reflects the extent of lipid peroxidation which inturn is a consequence of higher oxidative stress by various environmental stresses. Lower membrane stability has also been reported in susceptible genotypes of chickpea (Deb *et al.*, 1996).

5.1.3. Leaf water xylem potential (-MPa)

Leaf xylem water potential is considered as a good indicator of plant water status since it was associated well with the different plant functions and productivity in legumes (Phogat *et al.*, 1984). In the present investigation, xylem water potential provided good differentiation among the pulses genotypes. As shown by the TTC and EC methods, variety CO 4 was more tolerant than CO 2 in cowpea and CO 4 tolerant than VBN 1 in green gram. Soybean and other pulses had no significant differences between the varieties. Similar to cowpea and green gram a decrease in water potential was evident in flooded treatments. The above results were in line with the findings of Nelson *et al.* (1983). Decrease in water potential under stress reflects the increased hydrolysis of macromolecules into simpler ones like mono and disaccharides and amino acids especially proline resulting in higher osmolite concentration (Deb *et al.*, 1996).

5.1.4. Alcohol Dehydrogenase (ADH) activity

Alcohol Dehydrogenase was also taken as a selection criterion for screening pulses in the absence of oxygen. ADH is considered essential for anoxia survival, presumably because it recycles NAD^+ for continued glycolysis in the absence of oxygen (Russel *et al.*, 1990). From the present study, in green gram the variety CO 4 synthesized more ADH than VBN 1. In cowpea the variety CO 4 showed higher activity of ADH than CO 2. These results were consistent with published data in barley and rice by Wignarajah *et al.* (1976) which showed that flooding tolerance was associated with increased ADH activity. However other pulse varieties had no differences in their ADH activity. Also the above findings were corroborated by Liao and Lin (1995). Rapid increase in alcohol dehydrogenase was considered as a powerful adaptive mechanism for flooding tolerance in wheat varieties (Cao and Cai, 1991).

Our study showed that four methods could be used to evaluate the degree of stress imposed on pulse genotypes differentially sensitive to waterlogging. The TTC and EC methods were more time consuming and results were more variable. However, the other two methods *viz.*, the status of water potential and ADH activity require only 10 day old seedlings thus permitting the use of larger samples and more efficient use of space.

5.2. Glass House Study

5.2.1. Morphological parameters

5.2.1.1. Plant height

Height of the plant has been considered as an important morphological parameter related to growth and development of a plant. Flooding decreased plant height significantly with an increased flooding duration at all the stage of crop. In the present study, the plant height was significantly reduced due to flooding and the maximum reduction was recorded in 10 days of flooding. In cowpea, the variety CO 4 showed lesser decrease in plant height due to its tolerance nature to flooding situations. In soybean, both varieties had no differences in their plant height. However, in green gram CO 4 behaved as tolerant to the plant height. The impact of water logging in shoot growth can be observed on changes in growth habit, visual health, internal anatomy, water relations and hormonal and nutritional composition. Flooding at early growth stages decreased the plant height whereas tolerance increased as stage advances. These results corroborated the earlier reports of Jhorar *et al.* (1995) in mungbean, Sorte *et al.* (1995; 1997) in soybean and Goswami (1990) in cotton. Similar observations were also made by Sallam and Scott (1987) in soybean.

5.2.1.2. Root parameters

The stress experienced by roots in flooded or waterlogged soil intensifies with time. The well-established sensitivity of root extension to ethylene suggests a role for the gas in controlling extension growth under waterlogged soil. Flooding suppresses

the formation of root hairs and increased the branching of roots. According to Keelay (1979) the waterlogging tolerant ecotype when waterlogged partially loses its original roots while initiating more number of new roots that are more succulent, larger in diameter and more branched than non waterlogged roots. In present study, the tolerant genotypes showed more root length and numbers. The root numbers were larger at flowering stage than other stages but root length was more at later growth stages. The length of adventitious root was more when flooded for 10 days compared to 5 days. The above results were in accordance with the findings of Jhorar *et al.* (1995), Sorte *et al.* (1996) and Sallam and Scott (1987).

The production of adventitious rootlets is a frequent response of inundated plants promoted by ethylene under anoxia (Kawase, 1981). It is believed that they replace the function of the original roots and helps in the survival and recovery of the plant by providing water, nutrients and hormones and acting as sink for the assimilates from shoot (Jackson and Drew, 1984).

5.2.2. Growth attributes

5.2.2.1. Leaf area

Leaf area is one of the important photosynthetic parameters in crop plants, with a positive relationship to net photosynthetic activity. In the present investigation, among the three pulses, cowpea recorded more leaf area than soybean and green gram. In cowpea, the variety CO 4 showed maximum leaf area reduction than CO 2 under 10 days of flooding. However in green gram there was no differences between the varieties and in soybean the variety CO 1 had maximum leaf area than CO 2. Variation between species and stages of development may arise as a result of differences in growth and developmental process that occur at the times of waterlogging. In contrast, waterlogging at different stages resulted not only in reduced leaf expansion, but also in reduced stem extension and increased leaf senescence (Orchard and Jessop, 1984). The leaf area extension was markedly suppressed by waterlogging during flower bud differentiation. It is evident that the CO 4 in cowpea

and green gram possesses the ability to adapt for waterlogging. However, the soybean varieties did not differ in their behaviour to flooding treatments.

5.2.2.2. Specific leaf weight

To understand the physiological basis of yield, growth analysis is perhaps one of the best methods available. The application of growth analysis to crop focuses the attention on the assimilatory aspect of yield determination (Watson, 1952). SLW is the leaf dry weight per unit leaf area and is a useful morphological character which can be used as a selection criterion while evaluating the yield potential of crop plants (Dornhoff and Shibles, 1970). Specific leaf weight (SLW) progressively increased from vegetative stage to podfilling stage. In cowpea there were no significant differences between the varieties for their SLW. However, in soybean the variety CO 1 showed more SLW than CO 2. The variety CO 4 green gram had maximum SLW reduction for 10 days of flooding. Higher duration of flooding decreases the SLW in all pulse varieties. The above findings are in accordance with the results of Haloi (1989) in rice.

5.2.2.3. Specific leaf area

Specific leaf area (SLA) is a measure of leaf thickness, which varies with cultivar, leaf position, growth stage and environment. In the present study, SLA showed an inverse relationship with specific leaf weight, with maximum at vegetative stage and progressively decreasing upto podfilling stage. The longer duration of flooding decreases the SLA in all species. The variety CO 4 in cowpea, CO 2 in soybean and CO 4 in green gram had higher SLA under 10 days flooding. This may be due to its tolerance nature to flooding situations. Usha Rani (1979) and Dias-filho et al. (2000) in *Brachiaria* species obtained similar results. Weifengzhen *et al.* (2000) recorded decreased leaf area, leaf weight and SLW at grain formation and filling stages of wheat.

5.2.3. Dry matter production

Dry matter production is an important criterion as it determines the source sink relationship. The first prerequisite for high yields is an increase in the total dry matter per unit area. All pulses difference in Total Dry Matter Accumulation (TDMA) due to flooding at all growth stages. Longer duration of flooding decreases TDMA effectively. Among the growth stages, the vegetative stage had lesser TDMA during flooding conditions. Similar results were reported by Mukhtar *et al.* (1990) in corn. The varieties *viz.*, CO 1 soybean and CO 4 green gram recorded high TDMA under flooded conditions. Both had more biomass production than other flood susceptible varieties like CO 2 in soybean and VBN 1 in green gram. Better adaptability and stability in tolerant variety under higher flooding stress were bestowed by higher accumulation of dry matter in plant. Yadav and Saxena (1998) reported similar results in mungbean. During nine days of waterlogging, mungbean variety T 44 had maximum accumulation of drymatter per plant followed by susceptible genotypes pant M2 and Pant M1. The present results are in line with the findings of Sorte *et al.* (1996) in soybean and Lizaso and Ritchie, (1997) in corn.

5.2.4. Nodulation status of pulses

Waterlogging severely impairs root nodulation of legumes and inhibits nitrogen fixation by previously formed nodules, effects that may be explained entirely as consequences of O₂ starvation, CO₂ enrichment, or ethanol toxicity.

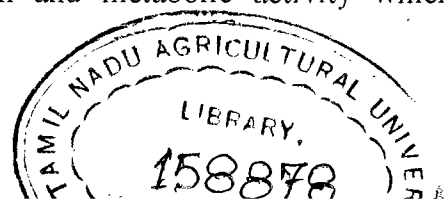
The efficiency of symbiotic nitrogen fixation in legumes is positively correlated with nodule number and nodule occupancy (Weber, 1966a). The present study revealed that flooding for 10 days at all growth stages reduced nodule number and nodule dry weights. No significant differences were observed in nodule number in all pulse varieties. Flooding at flowering stage completely inhibited nodulation in cowpea, soybean and green gram roots. This results supported by Minchin and Pate (1975); Andreeva *et al.* (1987) and Bishnoi and Krishnamoorthy (1992) in several legume species. Sallam and Scott (1987) suggested that the reduction of nodulation

was attributable to the suppression of root growth and root hair development in soybeans. In addition, owing to the formation of nodules may be reduced (Kozolwski and Pallardy, 1984). There are several reports corroborating the present study that nodulated grain legumes growing in waterlogged conditions generally fix less nitrogen and produce less drymatter and total nitrogen than in non waterlogged conditions. This phenomenon has been considered as a physiological one since oxygen transport to and within the nodule is impaired (Walker *et al.*, 1983).

The capacity of root nodules in pulses to survive and fix nitrogen in waterlogged soil is associated with the ability to develop a loose structure that facilitates gas exchange. The same results supported that the infected cells of submerged nodules had larger vacuoles than those from nodules of normally watered plants. It is suggested that this increase in vacuoles volume to protoplasm volume in infected cells may play a role in the tolerance of waterlogging in clover (Pugh *et al.*, 1995).

The assessment of biological nitrogen fixation and nitrogenase activity has been aided in recent years by acetylene reduction technique (Dilworth 1966). The present study showed that the nitrogenase activity declined in all pulses under flooded conditions. This may have been caused by the bacteroids of the curtailed carbohydrate metabolism of host tissue in the nodule (Huang and LaRue, 1985). However, the variety CO 4 cowpea and green gram recorded higher activity than other varieties. It was also found that activity declined significantly when water content exceeded field capacity and led to depressed aeration, as well as when the soil dried below field capacity (Sprent, 1972).

Reduced specific nodule activities may also be due in part to the concomitant decline in the content of leghaemoglobin, which facilitates the diffusion of oxygen from the nodule surface to the bacteriod. The anaerobic environment probably has lead to the arrest of nodule bacteriod respiration and metabolic activity which



eventually resulted in the depression of Leghaemoglobin (Lb) contents because the heme part required for the synthesis of Lb must be provided by the micro symbiont (Nadler and Avissar, 1977).

In the root nodules of leguminous plants, a variable diffusion barrier restricts the oxygen concentration in the cells but facilitation of its diffusion by leghaemoglobin and the high affinity for oxygen of the bacteroid cytochrome C oxidase allow the metabolism of both the infected cell and bacteroid to be aerobic under a wide range of external oxygen concentrations (Vance and Haichel, 1991). The rapid decrease in the intensity of colour is in accordance with the content of Lb estimated from nodules and the results unequivocally indicate the sensitivity of Lb components to excess water. The same results was observed in the present study also. In cowpea, the variety CO 4 showed less Lb content than CO 2 under 10 days of flooding. But as like that of cowpea, the green gram and soybean varieties had no significant differences for leghaemoglobin content. Among the growth stages, flowering phase showed higher Lb content. Similar results were also reported by Selvaraj and Karunambal (1995) in cowpea.

5.2.5. Physiological changes

5.2.5.1. Leaf gas exchange parameters

Leaf gas exchange parameters have been used to study the photosynthetic capacity of plants during flooding, except in some flood-tolerant plants that have developed adaptive mechanisms. So as to maintain better photosynthetic capacity (Topa and Cheeseman, 1992), flooding causes a significant decrease in the capacity for leaf gas exchange in most flood-intolerant plants like are *Triticum aestivum* (Trought and Drew, 1980a), *Lycopersicum esculentum* (Bradford, 1983), *Pisum sativum* (Jackson and Kzowalewska, 1983). In the present study, the photosynthetic rates and stomatal conductance decreased in all pulses under flooded conditions. The tolerant varieties had more photosynthetic rate and stomatal conductance than susceptible ones. The reduction of photosynthetic rate and stomatal conductance was

highest in longer duration of flooding. Similar results have been observed in tomato (Jackson, 1990a, 1990b) and apple (Jackson, 1991). In control and flooded plants, stomatal conductance were shown to be correlated with CER (CO₂ exchange rate) (Liao and Lin, 1985 in bitter melon; Vu and Yelenosky, 1991 in citrus). These results imply that stomatal aperture is a limiting factor for CER. The internal CO₂ concentration (C_i) of leaves was found to increase linearly with the duration of flooding, despite a reduction of stomatal conductance (Liao and Lin, 1994). Liao and Lin (2001) reported that stomatal aperture was not only limiting factor for CER but was partly responsible for the decrease in the photosynthetic capacity of mesophyll tissue. Also feed back inhibition of starch accumulation may result in a reduction of CER in flooded plants. All pulses had higher transpiration rates under flooded conditions. Jiang and Lenz (1995) reported that the increased transpiration during short term waterlogging were due to wide opening of stomata in strawberries. After 10 days, the stomata were closed and the leaves wilted; dark respiration exceeded net Pn.

Carbon assimilation and translocation are also reduced in plant sensitive to flooding. This is attributed, initially to stomatal closure but subsequently, changes in nutrient availability, accumulation of assimilates and hormonal imbalance are important causes for the reduction of photosynthesis in tropical forage grasses (Baruch, 1994).

5.2.5.2. Leaf water potential

Leaf Water Potential (LWP) was considered as a good indicator of plant water status since it was associated well with different plant functions and productivity in legumes (Phogat *et al.*, 1984). Flooding increases atmospheric humidity, which along with stomatal closure reduces transpiration. Stomatal closure in response to flooding was not induced by leaf water deficit. A root signal, probably a combination of reduced cytokinin synthesis and increased transport of ABA and ethylene, induces stomatal closure. Since transpiration was reduced during flooded conditions, the

amount of water uptake from roots need not be as high to maintain plant water balance as it would be in a nonflooded condition (Nilsen and Orcutt, 1996). In all pulses flooding treatments decreased the leaf water potential at all growth stages. There was no differences between the varieties for leaf water potential status. Present findings was in accordance with Singh *et al.* (1991) who reported that flooding affects Pn before stomatal closure and decline in leaf water potential in snap bean.

Flooding of pulses in present study did not resulted in a significant effect on the leaf water potential. The present results was corroborated with the findings of Gadallah (1995); Bishnoi and Krishnamoorthy (1992). Oosterhuis *et al.*, (1990) reported that when plants under high Vapour Pressure Deficit (VPD) were exposed to root hypoxia, there was a temporary decrease in leaf water potential. The timing of the changes in leaf water potential correlated with the initial reduction in stomatal closure in phaseolus seedlings (Neuman and Smit, 1991).

5.2.6. Chlorophyll fluorescence

Chlorophyll fluorescence has been dubbed as the plant physiologist stethoscope because they are extremely useful in the diagnosis of photosynthetic activity of plants under normal and stressed conditions (Krause and Weis, 1991). In the present study, the maximum total and variable fluorescence decreased under flooded conditions. The chlorophyll fluorescence ratio increased upto flowering stage only. The ratio of variable and maximum fluorescence indirectly proportional to the quantum yield of photochemistry and showed high degree of correlation to quantum yield of net photosynthesis of intact leaves (Krause and Weis, 1984). The varieties CO 4 in cowpea and green gram were tolerant in nature and soybean varieties were moderately tolerant to flooded stress. The tolerant nature might be due to slow reduction of chlorophyll fluorescence in leaves and the excitation energy was captured in PS II was high in flooded conditions. These were in accordance with the findings of Sharma and Singh (1994) in rice and Wullschelger *et al.* (1992) in soybean.

5.2.7. Biochemical Mechanism of flooding tolerance

5.2.7.1. Leaf soluble protein

Chloroplast contain approximately half of the total protein in leaves, and about one fourth to one half of their total protein is Rubisco and this enzyme accounts to one eight to one fourth of leaf protein.

The soluble protein content was estimated in order to find out the photosynthetic capacity of the varieties under the flood stress situation. Extent of variation in soluble protein content was well noticed in all the growth stages. Flooding reduced the soluble protein content in all pulses. The decline was associated with a concomitant decrease in NRase activity. However tolerant varieties (CO 4 in cowpea and green gram and CO 1 in soybean) which indicated the disruption of nitrogen metabolism, showed higher soluble protein content than susceptible ones. It was in confirmation with the findings of Sinha *et al.* (1995) and Sachs *et al.* (1980). Yu Qin and Rengal (1999) also reported that the decreased concentration of soluble protein in lupin and tobacco plants under flooding condition.

5.2.7.2. Root anaerobic proteins

Plants respond to flooding by altering the pattern of root protein synthesis. The protein, which was synthesized as specific responses to anaerobiosis were, called the anaerobic polypeptides (ANPs) or anaerobic proteins (Sachs *et al.*, 1980). In the present study the anaerobic root protein was significantly decreased with the increased duration of flooding at all stages. The flowering stage had more amounts of root anaerobic proteins. However, in all pulses the tolerant varieties accumulated more root anaerobic proteins than susceptible ones. Similar accumulation of root anaerobic proteins were also reported earlier by Kelly (1989) in maize root and Russell *et al.* (1990) in soybean roots. These proteins were necessary to maintain energy production under anaerobic conditions and also involved in glycolysis and their enhanced transcription serves to increase the capacity for glycolysis and fermentative respiration. It was in accordance with the findings of Bennett and Freeling (1987) who

reported a more complex pattern of proteins were synthesised under hypoxia than anoxia, some proteins were glycolytic enzymes, whereas others were hypoxic specific responses.

5.2.7.3. Chlorophyll pigments

Chlorophyll pigments play a major role in plant productivity as it is the only pigment which is responsible for photosynthesis. Thus, its destruction under flooding is deleterious to plant productivity. In many species grown in waterlogged soil conditions, premature chlorosis and senescence set in within a few days. In the current experiment, the maximum chlorophyll 'a', 'b' and total chlorophyll content recorded at flowering stage. The longer duration of flooding decreased the total chlorophyll, 'a' and 'b' contents drastically. During and following soil saturation or flooding (the period of flood stress) plants develop chlorotic leaf symptoms ie., characteristic of N deficiency stress. The development of chlorosis coincides with a decline in soil nitrate and total available soil N levels in soybean (Heatherly and Pringle, 1991). From the present study the variety CO 4 cowpea and green gram and CO 1 soybean were found to be tolerant due to its stability of total chlorophyll, 'a' and 'b' content. The stability of chlorophyll content for flood stress could be regarded as an index for tolerance, which might produce high photosynthetic efficiency and eventually showed higher yield. The above said observation was in accordance with Das and Sarkar (2001). Sinha *et al.* (1995) reported that the chlorophyll a/b ratio could be taken as an indicator of plant adaptation during anaerobiosis for their maximum energy production as well as for the generation of reducing equivalents, which could be later utilized for the biosynthesis of carbohydrate by reductive pentose phosphate cycle.

5.2.7.4. Total carbohydrates

Carbohydrates are the photosynthetic products in green plants and the ability of plants to accumulate carbohydrates with its partitioning into economically useful parts determine yielding ability. In the present investigation, data showed a decreasing trend of carbohydrate fraction in relation to different duration of flooding. The

tolerant varieties performed better over the susceptible by showing a less reduction of carbohydrate fractions over control. The reduction was more in susceptible varieties because of more utilization. This was in accordance with the reports of Wample and Davis (1983) and Barta (1987). Under root hypoxia, starch accumulation in leaves has been attributed to a reduced rate of translocation of carbohydrates from leaves to roots. This result was similar to that observed in flooded rice by Bertani *et al.* (1981) and Sarkar (1998).

5.2.7.5. Nitrate Reductase Activity (NRase)

Nitrate reductase is thought to play a key role in the regulation of assimilatory nitrate reduction (Sung and Sun, 1990). The present study revealed that flooding decreased the activity of nitrate reductase in all the pulse crops. Longer duration of flooding had detrimental effect than shorter duration of flooding. This might be due to the rapid decreased rate of synthesis of the enzymes. Reduced NRase activity may be attributed to the inhibition of enzyme synthesis and decreased availability of reductant. (Hoff *et al.*, 1992). Tolerant varieties showed more NRase activity than susceptible one. The marked reduction in NRase during flooding indicated a changes in metabolic activity especially protein metabolism in the leaves. It was also due to rapid depletion of the nitrate as oxygen was consumed by soil biota as anaerobic conditions develop. As a result, volatilization of soil by microbes and loss of nitrate were promoted through denitrification process (Kozlowski and Pallardy, 1984). Similar observation was also reported in wheat leaves by Youngping *et al.* (2000). Fewer nitrates taken up by the water logged soybean leaves in comparison with the controls was due to low oxygen effect on root metabolism (Van Toai and Bolles, 1991).

5.2.8. Ethanol Fermentation Enzymes-ADH and PDC

Among the biochemical changes observed in plants under flooding, a high level of fermentative metabolism in roots has been shown to be important for plant survival, because it supplies a high energy charge that can sustain metabolism in

soybean and rice roots (Jackson and Drew, 1984; Mohanty *et al.*, 1993). In plants, alcohol dehydrogenase (ADH) plays a central role in energy metabolism under conditions of low oxygen tension. When plants were subjected to hypoxic conditions such as waterlogging, carbohydrate metabolism switches from oxidative cycle (TCA cycle) to a fermentative mode (Sachs *et al.*, 1980).

Flooding stimulates the synthesis of a small group of proteins known as anaerobic peptides (ANP). All the characterized polypeptides were glycolytic enzymes in rice (Mujer *et al.*, 1993). Among the ANPs, ADH and PDC were predominant and have been extensively studied. ADH was considered essential for anoxia survival, presumably because it recycles NAD^+ for continued glycolysis in the absence of oxygen (Russel *et al.*, 1990). The present study revealed that data on alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) activity were increased by flooding at all the growth stages. Shorter duration of flooding (5 days) had showed higher activity of ethanol fermentation enzymes than longer duration of flooding (10 days). Similar observations were also reported by Pandey *et al.* (2000) in corn and Su and Lin (1996) in luffa roots. All pulses showed higher activity of ADH and PDC under flooding conditions. The variety CO 4 in cowpea and green gram had more fermentation enzymes activity than other varieties. These results were consistent with published data by Wignarajah *et al.* (1976) which showed that flooding tolerance was associated with increased ADH activity. This was in confirmation with the findings of McManmoom and Crawford (1971); Liao and Lin (1995) in bitter melon and Lin and Lin (1992) in wax apple. Alcohol dehydrogenase was responsible for the synthesis of alcohol and regeneration of NAD in alcoholic fermentation. The recycled NAD, enables glycolysis to continue under anoxia, thus producing a net 2 moles of ATP per mole glucose relative to the 38 moles of ATP produced under aerobic conditions through respiration (Davies, 1980).

Waters *et al.* (1991) and Setter (1993) presented convincing evidence that PDC could be rate limiting enzyme in alcoholic fermentation that controls the entry of

carbon into the pathway. Notably, the activity of PDC was usually many-fold lower than that of ADH, suggesting that the rate-limiting enzyme for ethanol synthesis in flooded roots was more likely PDC than ADH (Su and Lin, 1996).

5.2.9. Malate Dehydrogenase - Alternative fermentation pathway enzyme

One metabolic adaptation that plants could use to avoid the production of ethanol by the glycolytic pathway is to use an alternative route with an end product other than ethanol.

According to the metabolic theory proposed by Crawford (1967) and McManmon and Crawford (1971), flooding tolerance was achieved by minimization of ethanol production associated with re-routing from ethanol fermentation to malate production. Plants can utilize several different fermentation pathways that reduce NADH to NAD⁺. The activities of MDH (Malate dehydrogenase) were required for synthesis of malate (Nilson and Orcutt, 1996). In the present study, shorter duration of flooding (5 days) recorded higher activity of MDH than longer duration of flooding (10 days) at all growth stages. Flood tolerant varieties (CO 4 in cowpea and green gram and CO 2 in soybean) showed higher MDH activity than susceptible varieties. Malate dehydrogenase catalyzes oxaloacetate to malate allowing the regeneration of NAD, to maintain glycolysis. Malate was assured to be less toxic than ethanol. It was successful that any further conversion of malate to pyruvate and on to ethanol was prevented in flood tolerant species either by constitutive lack of appropriate enzyme (NADP - Malic enzyme) or inhibition of its actions (Crawford, 1971; Su and Lin, 1996 and Sevaram, 2000).

5.2.10. Hormonal regulation of morphological and anatomical changes

Progress in identifying roles for endogenous plant hormones in influencing morphological adaptations to poor aeration has been made in the following areas: (1) growth and internal morphology of flooded roots, (2) promotion of extension growth by stems of submerged species, and (3) morphological changes in shoots

where only the roots are flooded. The later responses necessarily involve internal transmission of one or more physiologically active "messages", between the stressed roots and the shoot system. Ethylene involvement in adventitious root formation and aerenchyma formation in several crops was summarized by Jackson *et al.* (1993).

Ethylene synthesis increases under flooded conditions when ACC synthase concentrations increase, which stimulates ACC synthesis. ACC then diffuses to aerated parts of the root and is converted into ethylene by ACC oxidase. Ethylene is far less soluble in water than in air. Therefore, more ethylene is retained inside plant tissues when flooding occurs and ethylene concentrations increase. Irrespective of the courses for elevated ethylene synthesis, it is important to know the important effect of ethylene on plants under flooded conditions. Increased ethylene is associated with adventitious root formation. Adventitious roots are probably formed in response to increased IAA concentration, but ethylene may make tissues more sensitive to the impact of IAA. Several lines of evidence support the present results. Voesenek *et al.* (1989) have suggested the following theory for adventitious root formation in flooded plants. During flooding the basipetal transport of auxin is inhibited, resulting an increase in auxin at the base of stem. High ethylene concentration makes the tissues sensitive to auxin, which stimulates hypertrophy and adventitious roots at the base of stem just above the waterline.

Plants exhibit a wide variety of morphological and anatomical responses to flooding of the soil. Some appear to have adaptive significance. In the present investigation, the flooded pulses exhibited pronounced basal stem hypertrophy (swelling of cells), adventitious roots and aerenchyma formation. In flood-tolerant varieties (CO 4 in cowpea and green) produced higher amount of adventitious roots than susceptible varieties. The adventitious roots were highly clustered together above the soil layer (negatively gravitropic) in irrespective of the varieties and presence of accelerated swelling of the stem. This was due to the result of accelerated lateral cell expansion and could be associated with increased intercellular space, cell lysis to form

an aerenchyma, and developed subsequent adventitious rooting. The increase in porosity of the stem could facilitate aeration of attached roots. Kawase (1981) provided evidence that enhanced ethylene concentration in the swelling tissue and increased cellulose activity may soften cell walls thus favoring hypertrophy. This was in accordance with the findings of Visser *et al.* (1996) who reported that a higher ethylene concentration in rumex species under flooding increases the sensitivity of adventitious root-forming tissues to endogenous auxins. Bacanamwo and Purcel (1999) evidence that flooding acclimation in soybean relying on N₂ fixation was dependent on the adventitious root and aerenchyma formation.

Changes in the root anatomy in response to flooding were closely correlated with changes in root morphology. After waterlogging old roots die, but numerous adventitious roots with well developed aerenchyma emerge from the base of the stem and grow to a limited extent into the anaerobic soil (Laan *et al.*, 1991), as it was also observed in the present investigation.

Adaptive mechanisms to secure a renewed supply of oxygen to flooded root tissues included the development of internal gas filled channels called aerenchyma, that allow oxygen movement from aerobic shoot to the anaerobic root (Jackson, 1989). Aerenchyma differs in origin among species and may be either lysigenous or schizogenous. Lysigenous aerenchyma develop as a consequence of senescence of specific cells followed by their autolysis and disintegration, whereas schizogenous aerenchyma develop by cell separation and cell division (Kawase, 1981). The microtomy section of flooded roots showed that aerenchyma proliferation was observed in the cortex of roots, especially in adventitious roots. The formation of aerenchyma in flooded pulse roots were normally accomplished by an extensive lysis of cortical cells through cell disintegration (lysigenous). The cortical cells of roots of non-flooded pulses were highly packed and no aerenchyma developed. The tolerant varieties like CO 4 in green gram and cowpea had extensive of aerenchyma development with more number of adventitious roots. Formation of gas spaces or

aerenchyma may be mediated by enhanced ethylene concentration under anaerobiosis in *Zea mays* (Konings, 1982). Similar results were also reported in summer leguminous crops (Shimamura *et al.*, 1996 and Mochizuki *et al.*, 2000). The resulted aerenchyma tissue benefits root aeration in several ways, but principally by opening up a conduit of small resistance to oxygen movement, from shoot tissues above the waterline, to the roots below (Armstrong, *et al.*, 1994).

Elevated ethylene content has also been associated with several other important adaptations of plants to flooded conditions. There existed a strong evidence that ethylene is related to the development of aerenchyma in many plants. In the present study, pulses produced lysogenic aerenchyma during flooding condition. More recent studies supported the present results that flooding induces ethylene synthesis and was related directly to aerenchyma formation in corn plants (Brailsford *et al.*, 1993). In addition, flooding and ethylene concentrations were correlated with an increase in cellulase activity. This may be significant for lysogenic aerenchyma formation, because cell wall breakdown may be needed to form intracellular space (Nilsen and Orcutt, 1996).

5.2.11. Free radical scavenging Enzymes

5.2.11.1. Catalase (CAT)

Catalase (CAT) is mainly involved in the decomposition of hydrogen peroxide and oxidation of hydrogen donors. Flood cause the impaired activity of catalase which leads to enhanced production of reactive hydroxyl radicals which inturn cause peroxidation of unsaturated lipids. In addition, structure and functions of enzymes and nucleic acids are also changed thereby structural integrity of membrane is affected. In the present experiment catalase activity increased in pulses under flooded conditions. The tolerant varieties CO 4 had maximum catalase activity than susceptible varieties. Five days of flooding recorded higher enzyme activities than 10 days of flooding in all pulses at different stages of crop development. CAT which localized in the peroxisomes of higher plants, functions in the decomposition of H₂O₂, which is

produced outside the chloroplasts by H_2O_2 - generating oxidases in the peroxisomes (Tolbert, 1971). Despite restricted localization, it may play a significant role in removing H_2O_2 (Bowler *et al.*, 1992).

5.2.11.2. Superoxide Dismutase (SOD)

In antioxidative systems of plants, SOD can remove O_2^- . As SOD may control other activated species (H_2O_2 , OH), it is defined as a key antioxidative enzyme in the system (Van Toai and Bolles, 1991). When O_2^- levels were elevated, the activities of SOD and other protective enzymes increased in tolerant varieties of pulses at 5 days of flooding in the present investigation. These results agreed with the reports of Pandey *et al.* (2000) and Crawford (1993). One probable explanation was that SOD enzyme may be induced by substrate O_2^- . Possibly, increased levels of active oxygen species stimulate the cellular protective mechanism to mitigate damage. It could be inferred that the reduction of SOD activity occurred before the H_2O_2 scavenging system in chloroplasts was decomposed. The lipid peroxidation and membrane damage in flooded corn leaves was induced mainly by O_2^- (Bin Yan, 1996).

5.2.11.3. Peroxidase

Peroxidase is an important enzyme involved in morphogenesis and auxin oxidation. It is the enzyme, which is very sensitive to environmental fluctuations being considered as a measure of plant resistance to stress. One protective system to flood tolerance involves SOD converting superoxide radicals to hydrogen peroxide, which is further reduced to water by peroxidase or catalase. In the present study, the flooding for 10 days showed higher activity of peroxidase than 5 days flooding in all pulses. These results were in accordance with the reports of Armstrong *et al.* (1994) and Monk *et al.* (1987). This finding suggests that under flooding, peroxidase enzymes could be induced to protect the cell metabolism from a subsequent exposure to O_2 and lipid peroxidation. However, the contradictory results reported by Devender Reddy and Mittra (1985) that peroxidase activity was lower in flood tolerant variety of rice.

5.2.12. Molecular mechanisms of flooding tolerance

5.2.12.1. Study of leaf and root protein profiles in pulses under flooded condition

The results of SDS-PAGE protein profile of pulses displayed a qualitative and quantitative differences in protein profiles between flooded and control treatments. The prominent expression of 98, 83 and 43 kDa protein in leaves of CO 4 (cowpea), 88 and 98 kDa in CO 2 (soybean) and 98, 88 and 14kDa in leaves of green gram varieties. In roots the variety CO 4 in cowpea, CO 4 and VBN 1 in green gram and CO 1 in soybean showed higher expression of 43 kDa protein under 5 and 10 days of flooding. The other anaerobic root proteins like 23 and 75 kDa showed a poor expression in flooded situation, while these proteins could be observed in control plants.

Plant roots respond to conditions of anoxia by arresting normal protein synthesis and preferentially synthesizing a set of polypeptides that are collectively called anaerobic proteins (Sachs *et al.*, 1980). These anaerobic proteins were either previously synthesized proteins gets expressed at an elevated level in response to the lack of O₂, or synthesized only under conditions of restricted O₂ supply. These proteins were enzymes involved in glycolysis and fermentation pathways, which provide the major amount of ATP synthesis under limited O₂ conditions when oxidative phosphorylation was arrested.

Flooding induced a similar pattern of altered gene expression as reported earlier by Hoffman *et al.* (1986) in barley roots and Kimmerer (1987) in Totton wood. The present results also corroborate the findings of Mujer *et al.* (1993) who reported that the tolerant species had polypeptides enhanced or repressed under flooded condition. Bennett Freeling (1987) identified a more complex pattern of proteins synthesized under hypoxia than anoxia, some protein were glycolytic enzyme, whereas others were hypoxia specific responses. The coordinate expression of anaerobic protein was shown to be accomplished by a common transacting factor that interacts with an anaerobic response element (ARE) in the promoter region of each

gene (Olive *et al.*, 1991). Flooding induced modifications by the protein components of the translational machinery, including differential phosphorylation of ribosomal proteins and initiation factors, which may play a role in the selection of translation of anaerobic messengers (Ricard *et al.*, 1994) as it was corroborated with the present investigations. Oxygen deficiency also selectively changes the translation of mRNA into protein. Drew (1990) reported that anaerobic mRNA were not translated under anaerobic conditions in plant roots, whereas those for ANP were translated, presumably reflecting the recognition of specific anaerobic signal on the mRNA. Finally the anaerobic mRNA was much more stable and had a longer half-life under oxygen deficiency.

5.2.13. Isozyme analysis

The present investigation was to understand the mechanism of flood tolerance at biochemical level by the antioxidant enzymes like peroxidase. The flooding for 5 days produced different isoform than 10 days flooding in all tolerant pulse varieties due to the decreased expression of peroxidase isoforms. The analysis of isozyme pattern of peroxidase revealed that the presence of specific isoforms in leaves and roots of different pulse varieties under flooded and control conditions. Differences in peroxidase isozymes were noticed among the pulses both in intensity and banding patterns. Multiple isoforms of peroxidase (POD) existed in many plant tissues as reported by Vanhuystee (1990). From the present study peroxidase seems to be sensitive to flood stress. In tolerant varieties (CO 4 in cowpea and green gram) the root expressed higher number of peroxidase isoforms compared to susceptible cultivars (CO 2 in cowpea, VBN 1 in green gram) which expressed less number of isoform patterns. However, in leaves there was no variation of isoforms pattern in all pulse varieties under flooded conditions. The results of the present investigation supported the earlier report by Zhang and Kirkam (1999) who reported that under water stress there was a potential increase in the POD activities, while it was decreased in maize (Wang *et al.*, 1995). This peroxidase enzyme may be used as a biochemical marker for evaluating the genotype to flood stress in pulses

5.2.14. Yield and yield parameters

The productivity of many agricultural crops were adversely affected when the soil is either temporarily or continuously flooded (Kozłowski, 1984). Flooding decreased the production of flowers and pods in all pulse varieties. Tolerant varieties had higher number of flowers and pod than susceptible varieties. The significant reducing effect of flooding was evident in pod yield per plant at all stages. Highest reduction in pod yield was noticed in 10 days of flooding compared to 5 days of flooding at flowering stage. Flooding for 10 days decreased the flower induction drastically when stress was applied at vegetative and early flowering stages. This was in conformity with the findings of Linkemer *et al.* (1998) in soybean. Present study demonstrated that pods per node could be used as criteria to quantify genetic and environmental responses to waterlogging. Krishnamoorthy *et al.* (1983) recorded a 56% reduction in the number of seeds produced in green gram when waterlogged for 12 days from three weeks after sowing. Waterlogging at the seedling, vegetative and preflowering stage decreased the numbers of pods per plant and waterlogging at pod filling stage decreased the proportion of flowering nodes that bear fruit in peas (Cannel *et al.*, 1979). Further they have also concluded that the flower production was determined at vegetative stage itself while pod development was predetermined at flowering stage.

Fertility coefficient is an important factor that correlates positively with the final yield. This predicts the number of pods produced out of total number of flowers formed in the plant. In the present study, the reduction in fertility coefficient was higher in flowering stage than other stages in all pulses. Longer duration of flooding had marked effect on fertility coefficient than shorter duration of flooding. This may be due to source limitation as evident from decreased leaf area, which hinder the conversion of flowers into pods. This was in accordance with the findings of Sorte *et al.* (1995) in soybean. The realization of full sink capacity depends upon the transformation of flowers into pods, which depends on the supply of assimilates from the source tissue.

21

The results on the number of seeds per pod, showed that flooding at podfilling stages had deleterious effect on filling of seeds in all pulse varieties. This might be due to the reduced seed size caused by flooding for 5 and 10 days. However, tolerant pulse varieties had more number of seeds per pod than susceptible one, because of the tolerant nature to flooding stress. Similar observations were also reported earlier by Griffin and Saxton (1988) in soybean.

Seed size plays a secondary role in explaining yield differences. The seed weight is decided by seed size and filling. The increase in the size of individual seed in turn increased the 100 seed weight. Present study revealed that significant variation in 100 seed weight was evident due to flooding stress and the highest decrease was obvious under 10 days flooding. The minimum 100 seed weight was noticed in 10 days of flooding treatment at pod developmental stage. Tolerant varieties of pulses had more 100 seed weight over susceptible ones. Reduced seed weight which may be due to reduced translocation capacity during pod filling stage has been shown to decrease seed size and was probably responsible for low seed size in the undrained site (Linkemer *et al.*, 1998). This was in support with the findings of Mochizuki and Matsumoto (1992) in soybean and Orchard and Jessop (1984) in sunflower.

Yield is the ultimate economic end product of crop productivity. The yield component relationship in the present study had brought out that the number of flowers and pods, fertility coefficient, number of seeds per pod and seed weight significantly influenced the yield under excessive soil moisture conditions. The present study revealed that severe reduction in seed yield was caused by 10 days of flooding, mostly due to ill effects on various yield contributing parameters like pod yield seeds per pod, seed weight etc. The results of Boem *et al.* (1996) and Heatherly and Pringle (1991) was in accordance with the present observations. They suggested that higher yield reduction was associated with longer duration of flooding which reduces all yield components drastically. The present data indicated that highest reduction in seed yield was due to flooding at flowering in all pulse varieties. This

was also in accordance with the findings of Sorte *et al.* (1997). Yield reduction caused by flooding at vegetative stage was due to entirely less number of flowers and reproductive nodes, where as reduced yield at flowering was mainly caused by lesser flower numbers and pods per reproductive node. Yield lose at pod filling was entirely related to decreased seed number and size in soybean (Linkemer *et al.*, 1998).

5.3. Chemical manipulation for induction of flooding tolerance in green gram cultivars

Flooded soil conditions can rapidly damage the vegetative growth of plants and then reduce the final economic yield of the crop. Symptoms of waterlogging injury are a reduction of shoot extension, accelerated senescence, epinastic leaf growth, adventitious root production at the base of the stem, an overall reductions in the rate of growth and in old plants, the premature abscission of leaves, flowers and fruits. Several of these responses are associated with changes in the production and translocation of hormones and thus finally affect the yield. Spraying plants with an appropriate hormone at low doses can often reproduce or overcome these effects. The deleterious effects of flood stress were due to anaerobic conditions and impaired respiratory metabolism with the resultant inhibition of water and mineral uptake. A decline in the synthesis or uptake of indoleacetic acid (IAA), gibberellin and cytokinin and more production of Abscisic acid (ABA) and ethylene by roots were the consequences of flood stressed plants (Zhang and Davies, 1987). Some of the harmful effects of flooding that were reported earlier relieved by the application of plant hormones in tomato (Jackson and Campbell, 1979) and growth retardant in gram (Krishnamoorthy *et al.*, 1983). In the present study, number of flowers produced per plant decreased under flooded conditions. Abscission of flowers might be one of the reasons for the lowest yields. The deleterious effect of flood was more marked at flowering followed by pod-filling stages. Application of ameliorants significantly increased the number of flowers when compared with the unsprayed plants in green gram varieties, the effect being more in VBN 1 than CO 4. Application of brassinosteroids at 0.1 ppm and salicylic acid (100 ppm) showed lesser reduction in

number of flowers when compared with other treatments. This was in agreement with earlier results obtained by Raskin (1992) and Adam (1994). Leslie and Romani (1988) pointed out that application of salicylic acid influences flower stimulation and strongly inhibited the endogenous ACC-stimulated ethylene productions.

The present study showed that pod number per cluster and per plant was significantly decreased by the flooding stress, as pod number is considered to be a major yield determinant. This was in accordance with the work of Linkemer *et al.* (1998) and Sorte *et al.* (1996). Among the varieties, CO 4 produced more number of pods than VBN 1. The per cent reduction in pod number due to flood stress was more in VBN 1. Among the various growth hormones, the plants sprayed with brassinosteroids, salicylic acid and gibberellic acid showed more number of pods per cluster and per plant at flowering stages. This was in conformity with the work of Gomathi (1996) who noticed that brassinosteroid application in soybean increased the seed and pod number through an increase in fertile node number and number of pods per fertile node.

Source activity influences the fertility coefficient. The fertility coefficient showed a significant positive relationship to yield. In the present investigation flooding decreased the ratio of fertility coefficient significantly. It may be due to the reduction in flower production and nitrogen supply to the developing pods and seeds.

The first prerequisite for higher yields is an increase in the total dry matter per unit area. Generally, plant biomass decreased under waterlogged conditions. In present study, the variety CO 4 had higher biological yield than VBN 1. This was supported by the findings of Laan *et al.* (1991) then reported that flood tolerant varieties had more biomass production. The probable reason is increased source activity and better translocation of assimilates to the sink. Exogenous application of some ameliorants to flooded plants had some beneficial results. In present study, the salicylic acid (100 ppm) sprayed plants had higher biological yield followed by

202

brassinosteroids (0.1 ppm) and benzyl adenine (1 and 10 ppm) sprayed ones. This was in line with reports of Drew *et al.* (1979) in barley plants.

The variety VBN 1 had highest ratio of fertility coefficient. Among the treatments imposed the plant sprayed with foliar application of brassinosteroid (0.1 ppm) at flowering stage showed highest ratio of fertility coefficient followed by benzyladenine (1 and 10 ppm), salicylic acid (100 ppm) and gibberellic acid (1 and 100 ppm). The increased pod set attained due to the application of chemicals. This might be attributed to the ameliorants increased source activity at flowering stage and then translocated the required assimilates for pod development. This was in accordance with the findings of Singh and Kaur (1980) and Bhatia and Kaur (1997) in mung bean.

The data on number of seeds per pod indicated that impact of growth promoting phenomenon on the number of seeds per pod. Flood stress significantly decreased seed number and seed weight in all pulses. In general, seed weight was decided by seed size and seed filling activity. In the present study, maximum seed number per pod and 100 seed weight was recorded in the variety CO 4. The ameliorants salicylic acid, brassinosteroid, gibberellic acid and benzyladenine were on par with each other for seed number and seed weight.

Yield is a complex factor, which is determined by a definite sequence of developmental process. Both the number of flowers and pods besides seed weight are the primary yield components in pulses. Present study revealed that flood stress reduced the seed yield in green gram varieties and yield reduction was more in VBN 1 than CO 4.

The present study revealed that the spraying of brassinosteroids or salicylic acid increased the maximum seed yield in both varieties followed by benzyladenine under flooded conditions. Yield increase was due to the increased number of flowers,

22

pods, fertility coefficient, number of seeds and seed weight in brassinosteroid and salicylic acid sprayed varieties. It may be due to the increased photosynthetic efficiency by stabilization of chlorophyll, higher production and translocation of organic material from source to sink in the sprayed varieties. Brassinosteroids were known to regulate cell division, cell expansion, reproductive and vascular development, retard leaf abscission and enhanced resistance to stress (Adam, 1994). Ramaraj *et al.*, (1997) reported that homobrassinolide was most effective for improving yield of various crops subjected to flooding.

The main factor responsible for the lower seed yield in legumes is their poor harvest index and net photosynthetic limitation. In present study, the variety CO 4 recorded higher harvest index than VBN 1 under flood stress. Application of ameliorants significantly increased the harvest index in all the pulse varieties during flooding condition. Application of salicylic acid and brassinosteroids increased the harvest index in CO 4. This was in accordance with the results reported by Gomathi (1996) in soybean, Vardhini and Rao (1992) in groundnut and Sairam (1994) in wheat. Application of ameliorants had increased the HI due to greater translocation of photosynthates and also by increasing the sink intensity which was lower under flooded situation.

With the encouraging results from the present study of screening pulses for flooding tolerance, it was concluded that the variety CO 4 cowpea, CO 4 green gram and CO 1 soybean have adapted to grow in excess soil moisture conditions (for 5 days). To reduce flooding damage in pulses it was recommended that flooding at flowering and pod filling should be avoided. From the results of the experiment on induction of flooding tolerance by chemicals and plant growth regulators, it was concluded that the salicylic acid (100 ppm) and brassinosteroid (0.1 ppm) at flowering and pod filling stage was the best among all the treatments. These ameliorants will partially alleviate the deleterious effects of flood stress.

Summary

CHAPTER VI

SUMMARY

Flooding of soil is the most serious factor that limits pulses productivity. The present investigation was carried out with the following broad objectives:

- ❖ to screen the genotypes of pulse for flooding tolerance
- ❖ to understand the physiological, biochemical and molecular mechanisms of flooding tolerance
- ❖ chemical manipulation (chemicals and plant growth regulators) for induction of flooding tolerance in pulses.

Different pulse crops collected from Department of pulses, Tamil Nadu Agriculture University was subjected to laboratory screening for flooding tolerance. They were : (i) Triphenyl Tetrazolium Chloride (TTC) reduction method, (ii) Electrical Conductivity (EC) Test (iii) Pressure Chamber (PC) method (xylem water potential of leaves) and (iv) root Alcohol Dehydrogenase (ADH) activity. The tolerant and susceptible pulse genotypes were used in the pot culture experiment for anatomical physiological, biochemical and molecular mechanisms of flooding tolerance. Finally, an attempt was also made to identify a suitable ameliorants for induction of flooding tolerance and to improve yield under flood stress. The results of the various experiments are summarised below.

Among the different pulse genotypes, the tolerant genotypes had higher TTC reduction ability, membrane integrity, leaf water potential and root ADH activity. The tolerant pulse genotypes were CO 4 cowpea, CO 4 greengram and CO 1 soybean. Susceptible pulse genotypes were CO 2 cowpea, VBN 1 green gram and CO 2 soybean.

Under pot culture experiments, investigations were concentrated to study the morpho physiological, biochemical and molecular mechanism of flooding tolerance. Flood stress in vegetative or flowering or pod filling stages adversely affected all the physiological, biochemical, anatomical and yields components in pulses. Longer duration of flooding (10 days) was more detrimental to pulses than shorter duration of flooding (5 days). The intensity of the adverse effect due to flood stress was quite high at flowering and pod filling stages and hence these stages could be considered as the most critical stages for pulse growth.

In general, tolerant varieties performed better than susceptible varieties for all the parameters and expressed acquired tolerance. Morphological parameter *viz.*, plant height was much-reduced in susceptible genotypes under flooding. On the other hand root parameters like root number and root length had increased under different durations of flooding. The length of adventitious root was more at 10 days of flooding compared to 5 days flooding.

The biometric characters *viz.*, leaf area, specific leaf weight and specific leaf area were also found to be affected by flood stress. The degree of reduction was low in tolerant varieties (CO 4 cowpea and CO 4 green gram and Co 1 soybean) and high in susceptible varieties (CO 2 cowpea and VBN 1 green gram and CO 2 soybean). Variation between species and stages of development was noted as a result of differences in growth and developmental process, which occurred at the times of flooding. The tolerant varieties have produced high biomass than susceptible ones. Better adaptability and stability of the tolerant variety under flood stress appeared to be the reason for higher biomass production.

Flooding severely impaired nodulation of pulses due to O₂ starvation. Flooding at flowering completely inhibited the nodule activity of pulses. Flooding after nodulation has reduced the nitrogen fixation, dry matter production besides showing low nitrogenase activity and reduced leghaemoglobin content. Tolerant pulse varieties

had a capacity to survive and fix nitrogen in flooded soil in association with the loose structure developed in nodule that facilitates gas exchange. Leaf gas exchange parameters like photosynthesis and transpiration rates had decreased in all pulses under flooded condition. The tolerant pulse varieties had more photosynthesis and transpiration rate than susceptible one. Leaf water potential decreased in all pulse varieties at all growth stages.

The chlorophyll content 'a' and 'b' and total were found to be reduced under soil flooding for almost all pulse varieties. Tolerant varieties CO 4 cowpea and greengram and CO 1 soybean showed higher chlorophyll contents than susceptible ones. Chlorophyll fluorescence ratio decreased under excessive soil moisture conditions. In cowpea and green gram the variety CO 4 had expressed tolerant nature for chlorophyll fluorescence ratio.

Reduction in soluble protein content under flooding was due to impaired synthesis of amino acid and nucleic acids. Tolerant varieties showed higher soluble protein content and NRase activity than susceptible ones.

Anaerobic root protein was increased under flooded condition. In all pulses the tolerant varieties accumulated more root anaerobic proteins than susceptible ones. These proteins are glycolytic enzymes, which maintain energy production under anaerobic conditions.

Reduction of total carbohydrates and starch under flooding showed impaired synthesis and translocation of carbohydrate fraction. The reduction was more in susceptible varieties.

Flooding stimulated the synthesis of a small group of proteins known as anaerobic polypeptides (ANPS). These were glycolytic enzymes *viz.*, ADH and PDC. Tolerant varieties had more activity of these fermentation enzymes than susceptible

varieties. Both ADH and PDC were considered essential for anoxia survival because it recycles NAD^+ for continued glycolysis in the absence of oxygen.

Shorter duration of flooding showed higher activity of MDH than longer duration of flooding at all the growth stages. Flood tolerant varieties had higher MDH activity than susceptible varieties. MDH reduced oxaloacetate to malate, thus producing NAD to maintain glycolysis and minimised the ethanol production.

Ethylene synthesis was increased under flooded condition in all the pulse varieties whereas tolerant varieties accumulated more ethylene content than susceptible ones. The low oxygen partial pressure stimulated biosynthesis of ethylene by increasing ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity, thus further enriching the stem and roots with ethylene.

The flooded pulses produced stem hypertrophy, adventitious roots and aerenchyma formation whereas tolerant varieties had produced higher amount of adventitious roots and accelerated swelling of the stem.

Microtomy section of flooded roots revealed that aerenchyma was formed in the cortex of roots especially in adventitious roots. Tolerant varieties had larger internal gas filled spaces in adventitious roots.

Activities of antioxidant enzymes *viz.*, CAT, POX and SOD increased under flooded condition. The tolerant pulse varieties had maximum antioxidant enzymes than susceptible ones. One protective system for flood tolerance involves SOD converting free oxygen radical to H_2O_2 , which further reduced to water by peroxidase or catalase.

Electrophoretic study of protein profile in leaves and roots of pulses showed a quantitative difference between flooding and controlled conditions. In leaves, the

22

prominent expression of 43 kDa protein in all the pulse varieties was noticed. The anaerobic root protein like 23 and 75 kDa revealed a poor expression in flooded condition. The tolerant species had polypeptides and were enhanced or expressed under flooded condition.

Isozyme banding pattern of POX revealed the presence of distinct isoforms with polymorphic nature under control and flooded conditions. In roots, POX isoforms showed distinct difference between tolerant and susceptible pulse varieties. In leaves there was no variation of isoforms pattern in all the pulse varieties tested under flooded situation. Presence of this POX isozyme could be used as a biochemical trait for screening pulses to flood stress.

Flooding decreased the production of flowers and pods and further reduced the fertility co-efficient. During flowering highest reduction was observed at 10 days of flooding than at 5 days flooding. Ultimately number of seeds per pod and 100 seed weight were reduced under flooding condition. Thus all reduced yield components affected the final seed yield. Flooding at flowering and pod filling was most vulnerable than at vegetative stage. The deleterious effect of flood was more marked at flowering followed by pod filling stages. The inhibitory effect of 5 days of flooding was partially alleviated by different ameliorants at flowering and pod filling stages.

Among the ameliorants tested, salicylic acid (100 ppm) and brassinosteroid (0.1 ppm) as a foliar spray at flowering and pod filling stages edged out other spray chemicals and recorded higher values of all the yield components viz, flowers per plant, pods per cluster, pods per plant, fertility co-efficient, seeds per pod, 100 seed weight, seeds per plant and harvest index.. The yield increase due to ameliorants was brought about both by increased yield components and dry matter accumulation. The correlation between yield component and seed yield was significant.

Comparison of two varieties (CO 4 and VBN 1) in green gram showed that the variety CO 4 was respond well to the application of ameliorants under flooded conditions.

From the findings of the present investigation it is evident that flooding duration longer than 5 days should be avoided if maximum response to irrigation is to be achieved. To reduce flooding damage in pulses it was recommended that flood irrigation at flowering and pod filling should be avoided. In addition, spraying pulses with appropriate ameliorants *viz.*, salicylic acid (100 ppm) and brassinosteriod (0.1 ppm) at sensitive growth stages also partially alleviate the deleterious effects of flood stress.

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Plates

Plate.1. View of pot culture experiment



Plate.2. Chemical manipulation for induction of flooding tolerance in green gram



Plate.3. Formation of adventitious roots and stem cracking in pulses under flooded conditions

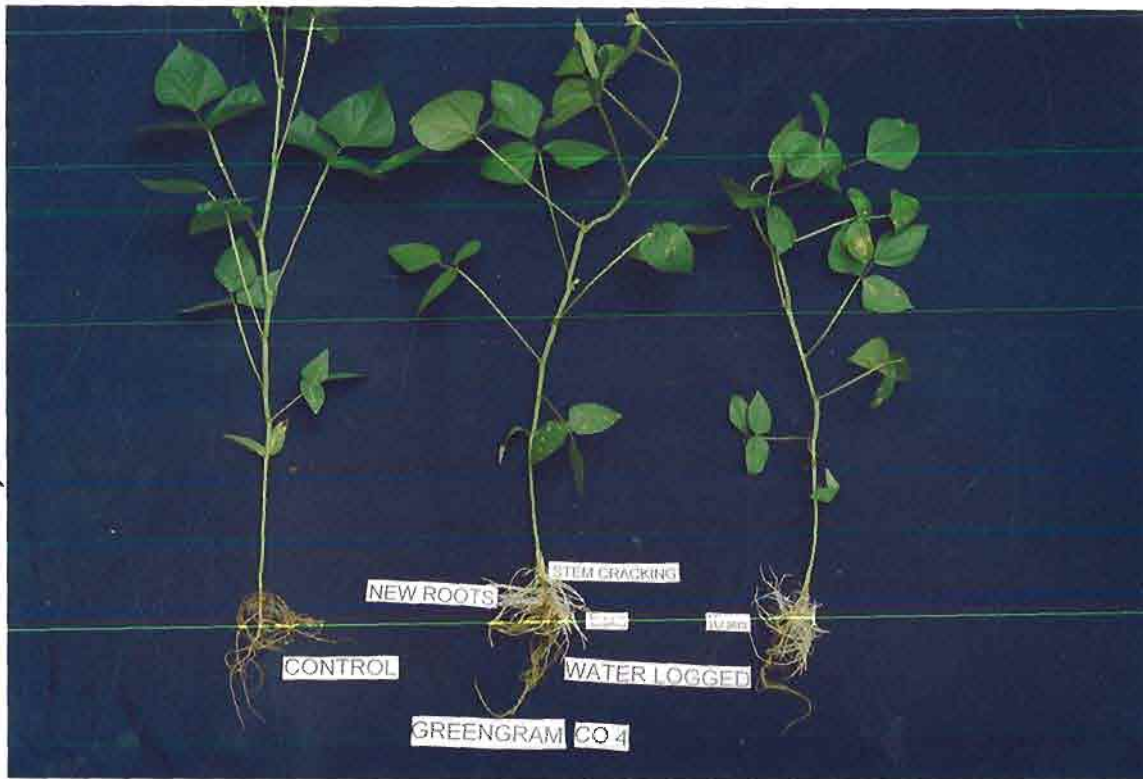


Plate.4. Formation of adventitious roots and floating roots in pulses under flooding conditions



Plate.4. Formation of adventitious roots and floating roots in pulses under flooding conditions

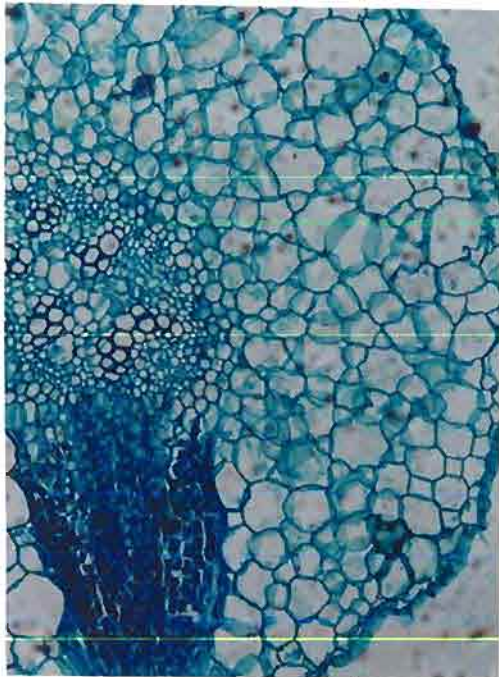


Plate.4. Formation of adventitious roots and floating roots in pulses under flooding conditions

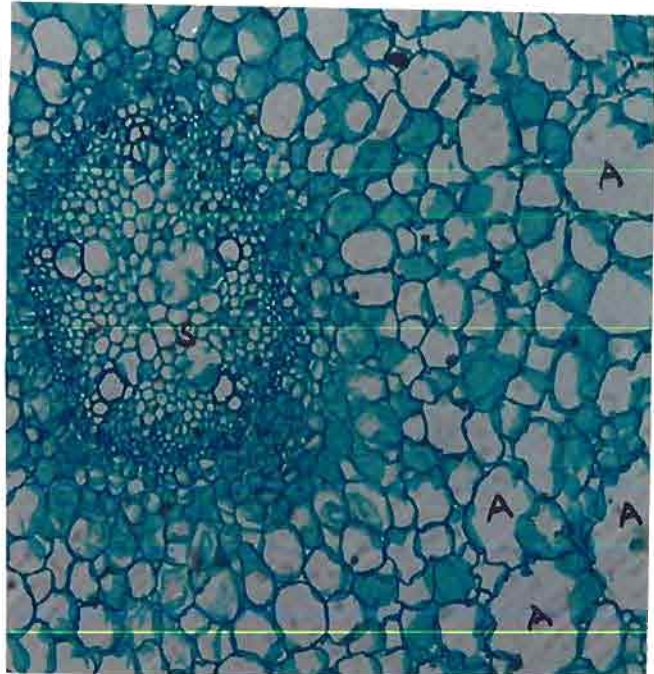


Plate 5 Microtome section of adventitious roots of pulses grown in control and flooded condition

Cowpea - CO 4

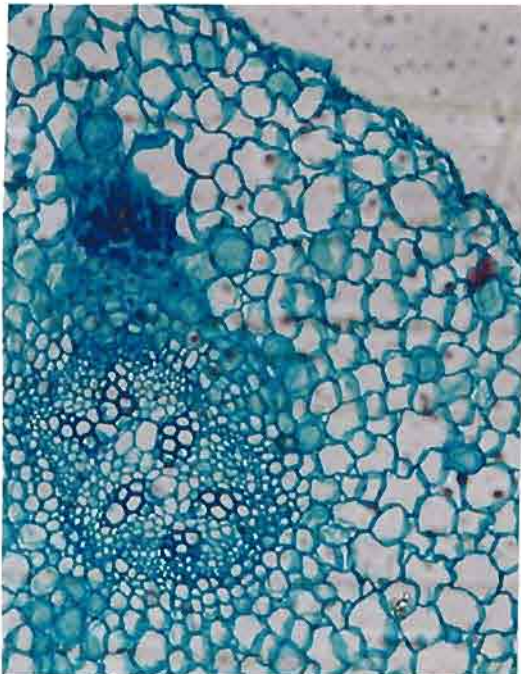


control

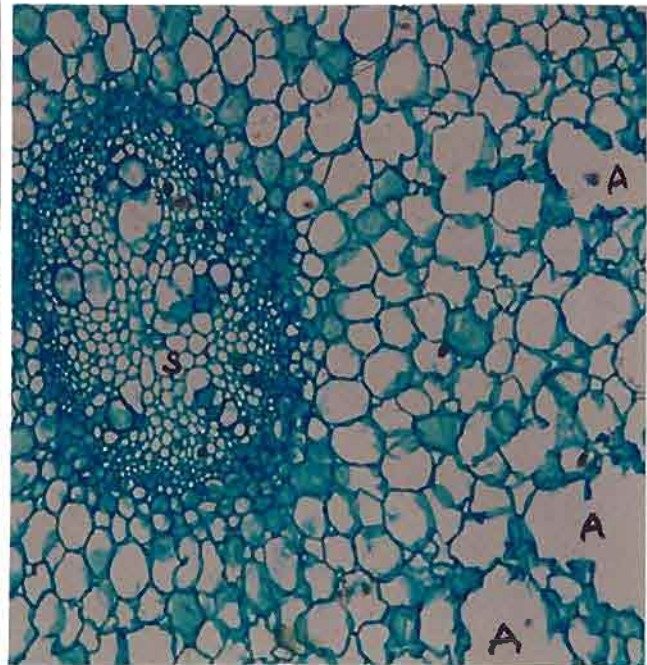


flooded

Cowpea - CO 2



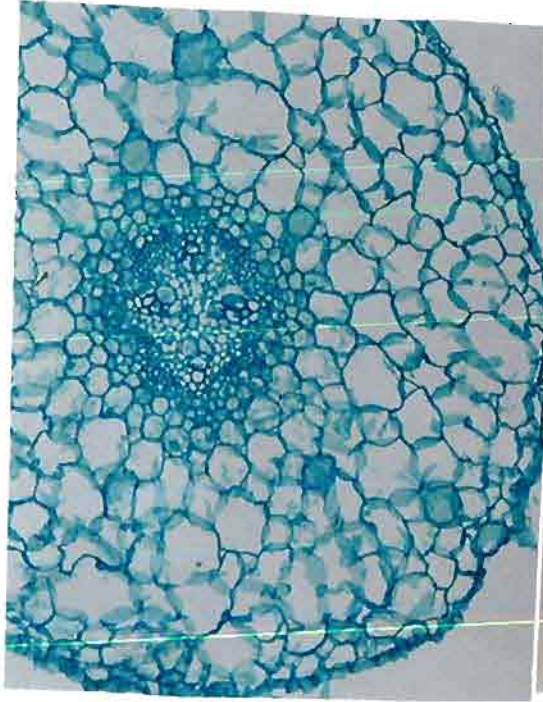
Control



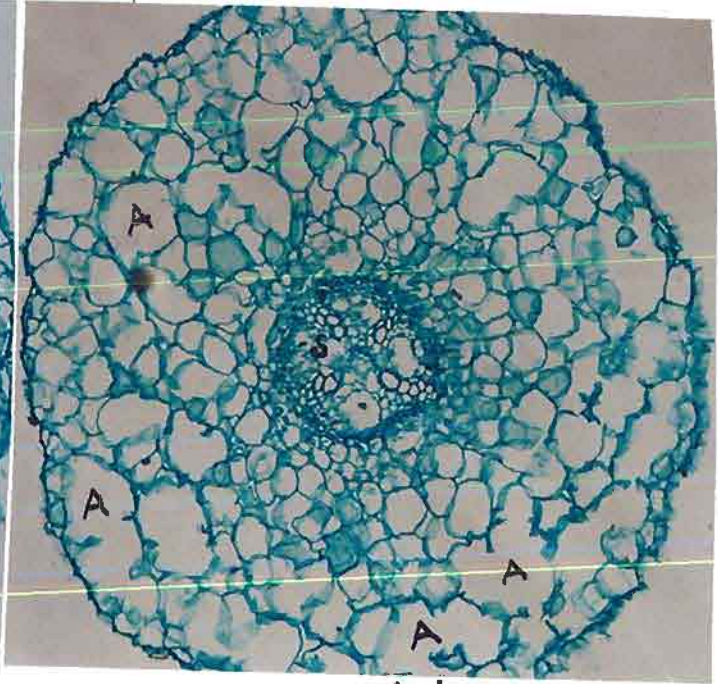
flooded

A - Aerenchyma
S - Stele

Green gram - CO 4

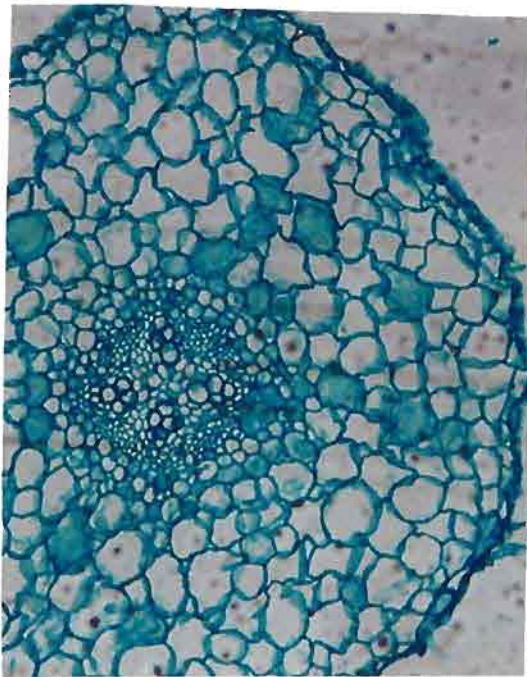


control

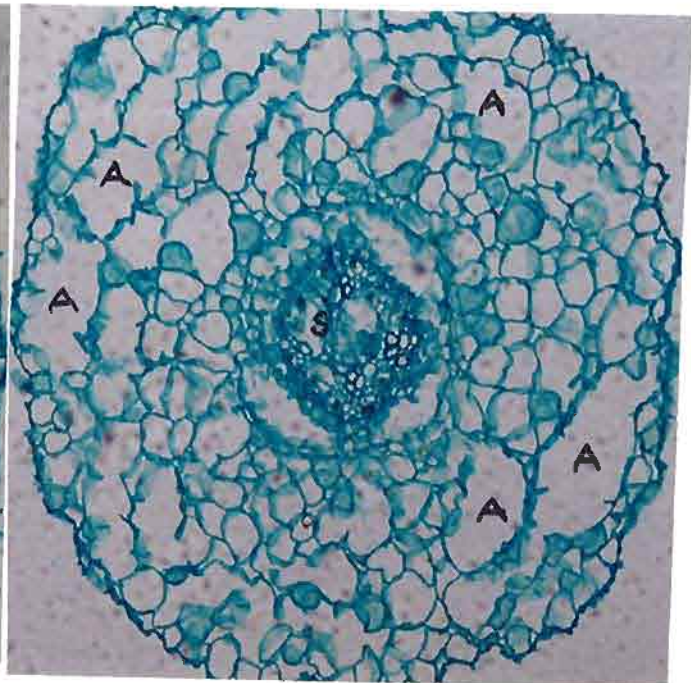


flooded

Green gram - VBN 1



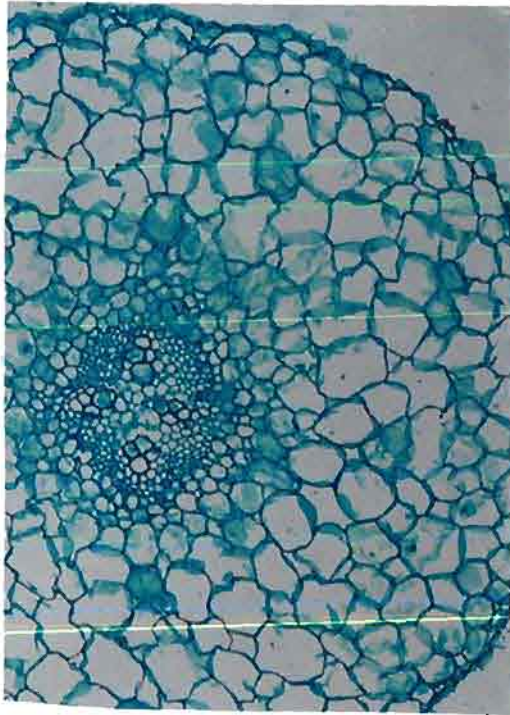
control



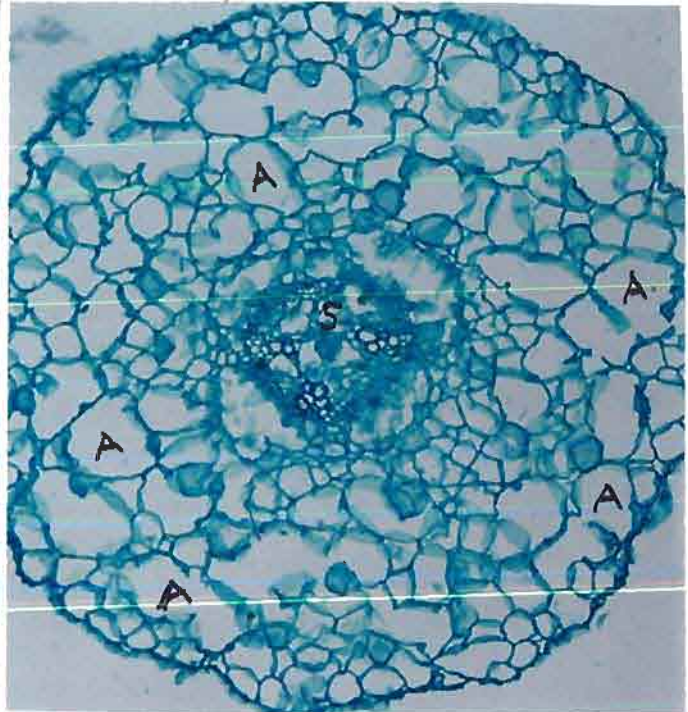
flooded

A - Aerenchyma
S - Stele

Soybean - CO 1

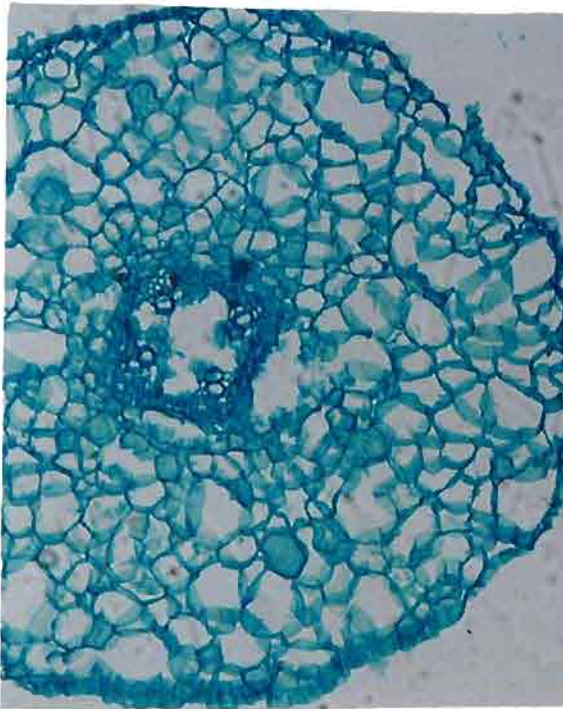


control

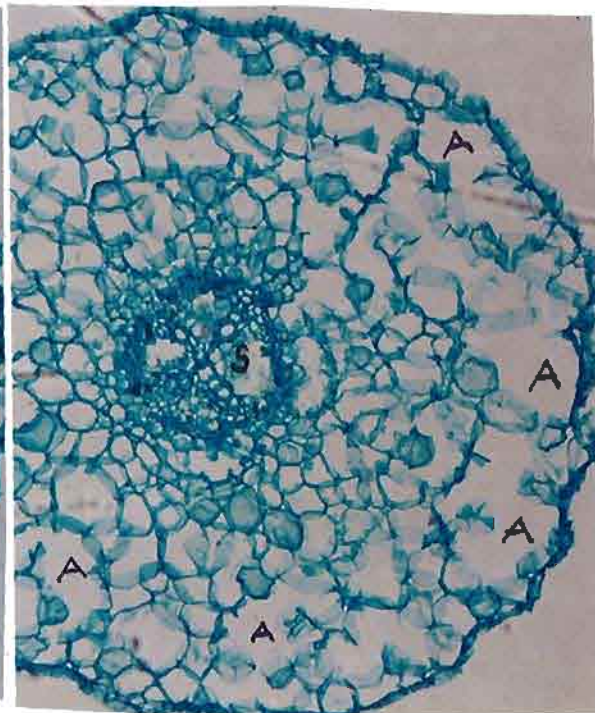


flooded

Soybean - CO 2



Control

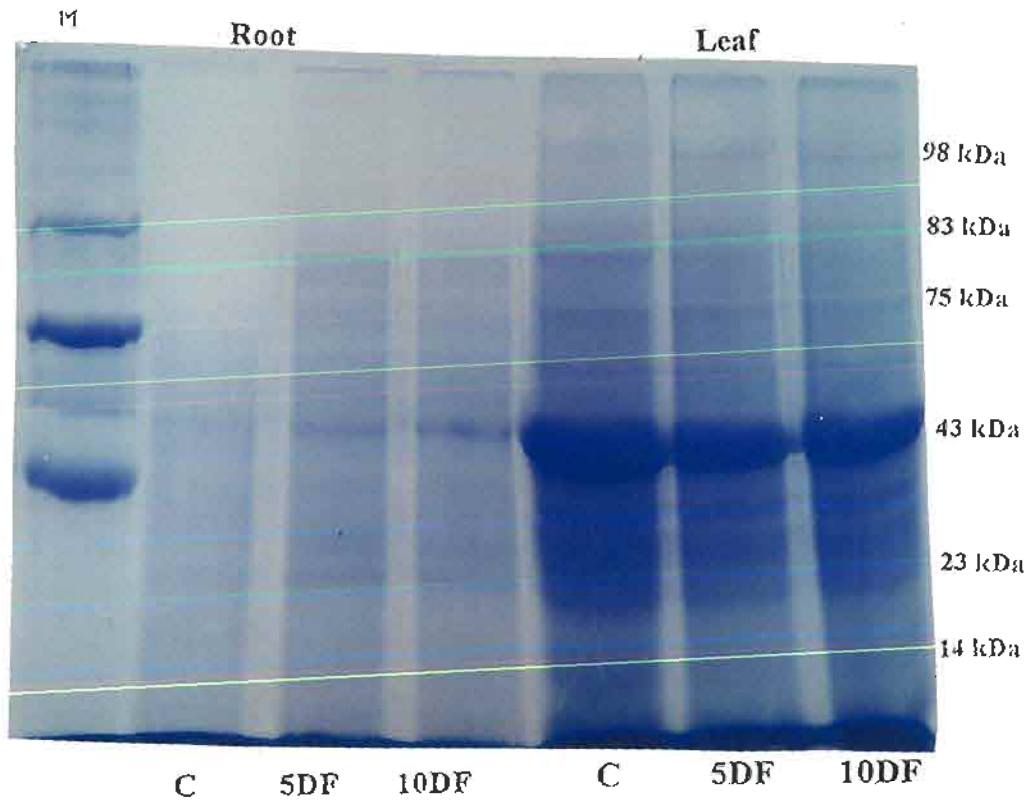


flooded

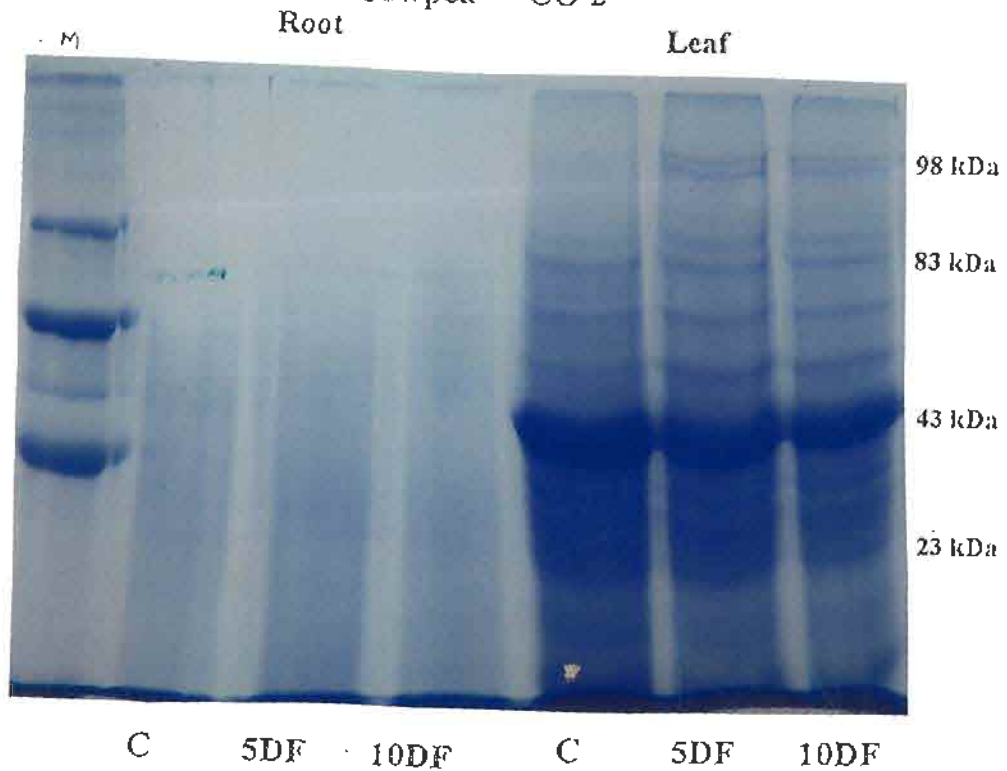
A - Aerenchyma
S - Stele

Plate.6. Leaf and root protein profiles (SDS-PAGE) of pulses under control and flooded conditions

Cowpea - CO 4



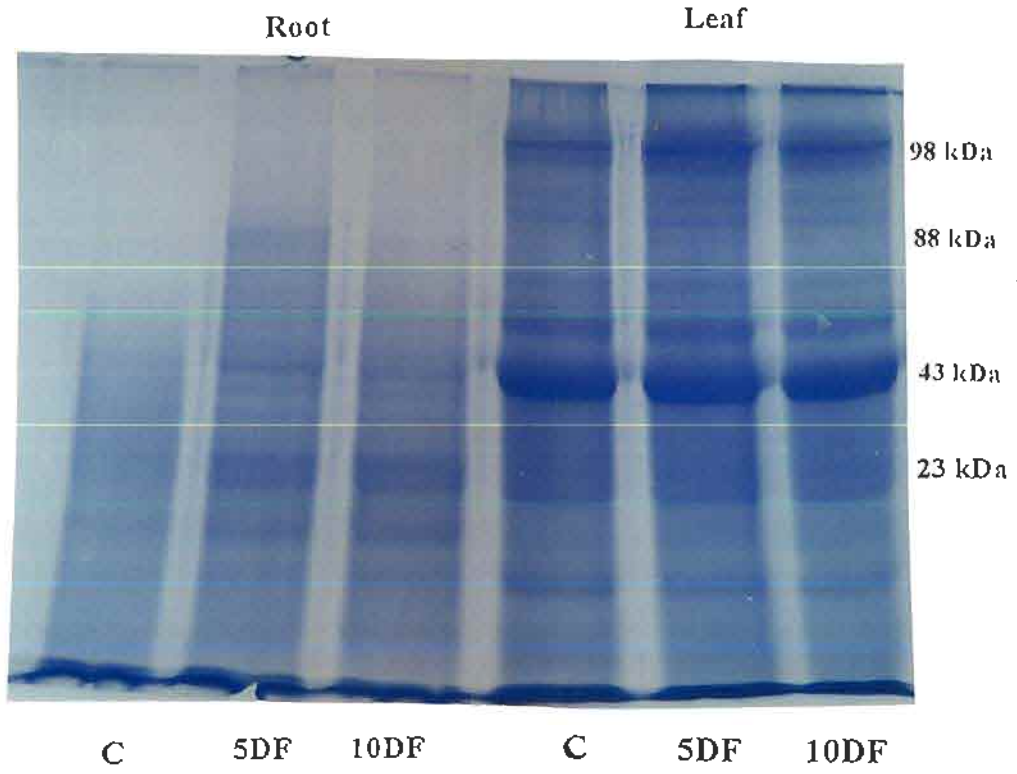
Cowpea - CO 2



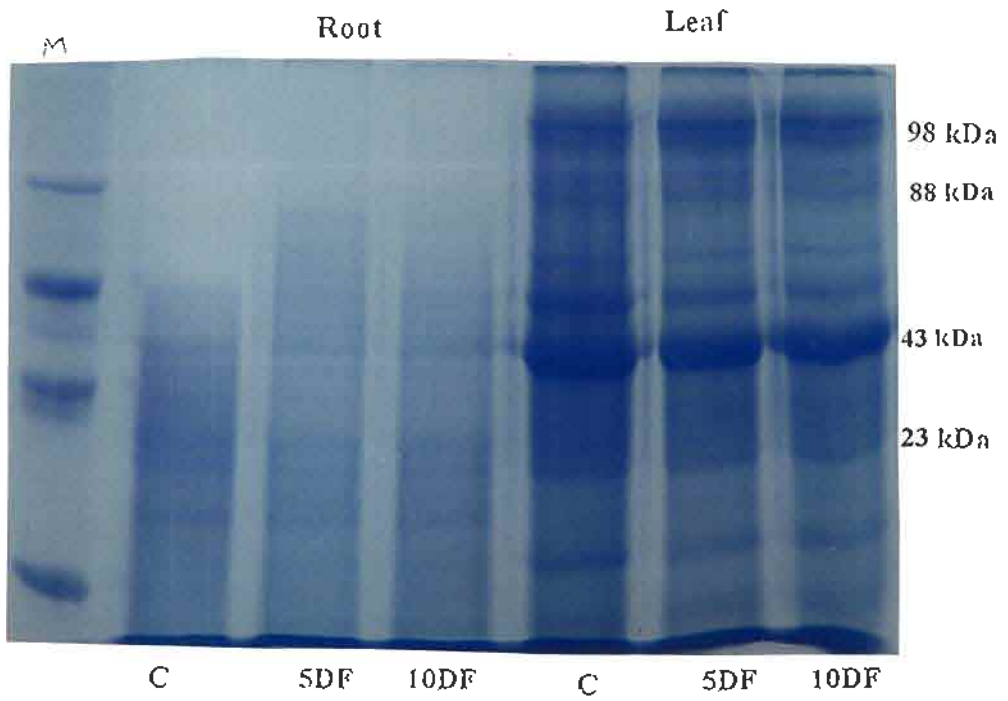
C Control
5DF 5 days flooding
10DF 10 days flooding

26

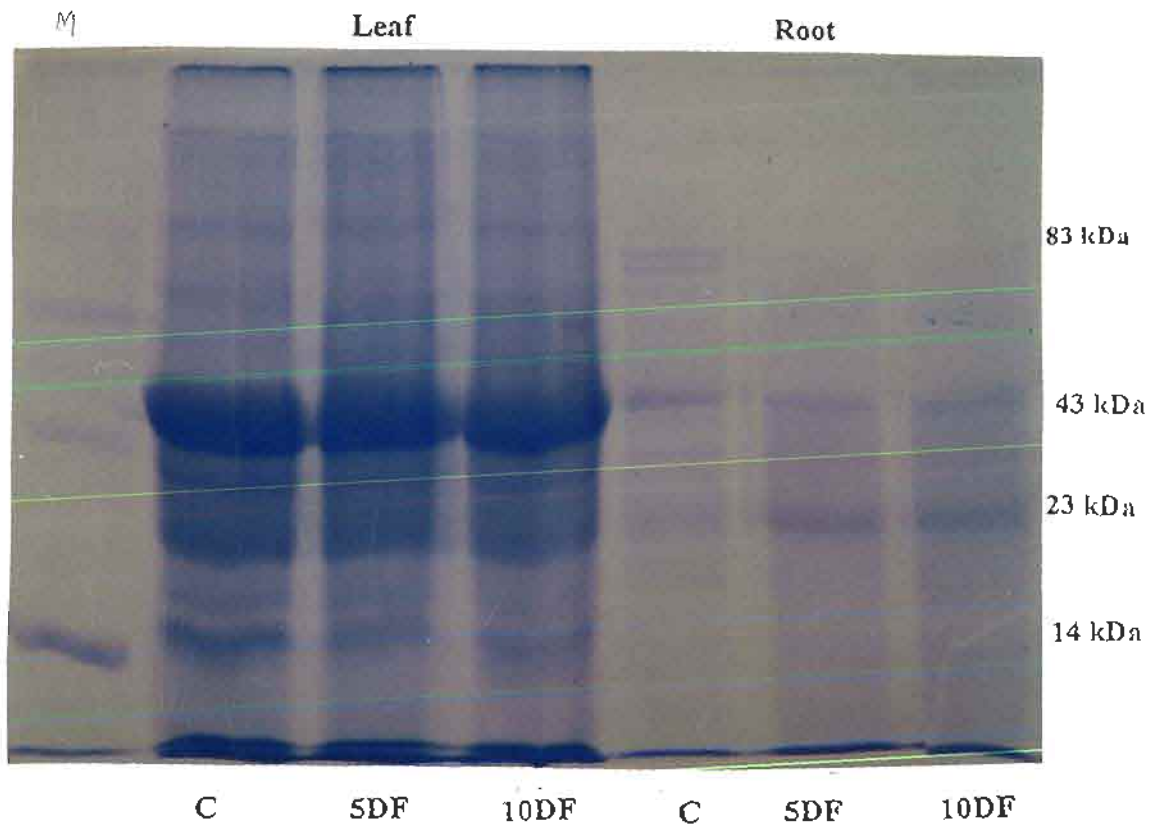
Soybean - CO 1



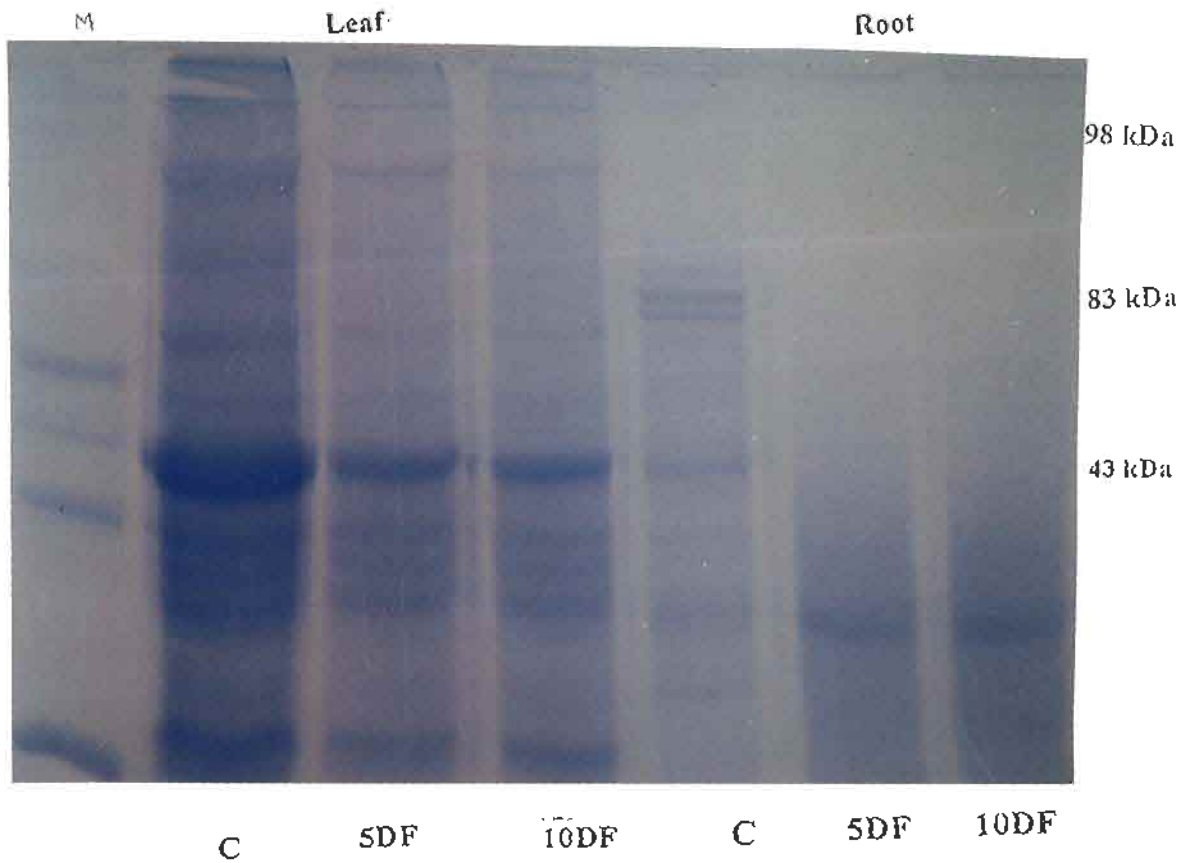
Soybean - CO 2



Green gram - CO 4



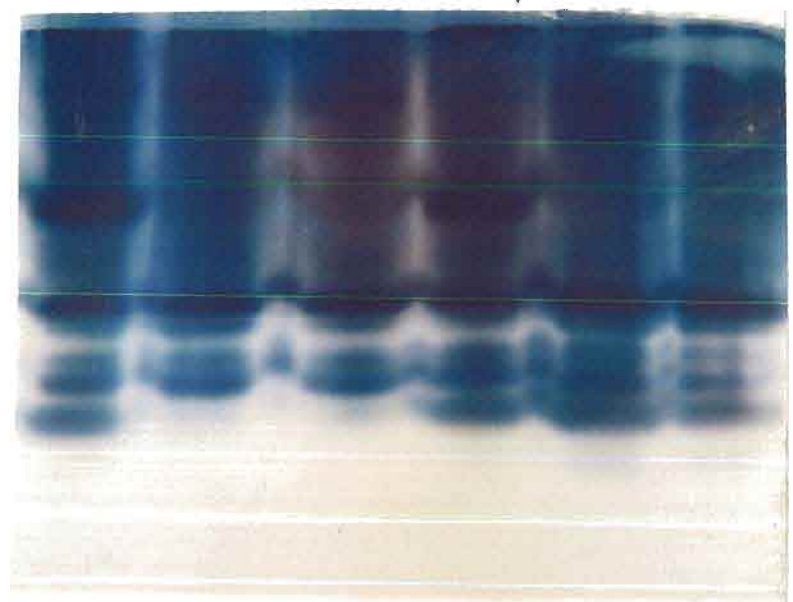
Green gram - VBN 1



Soybean - Root

CO 2

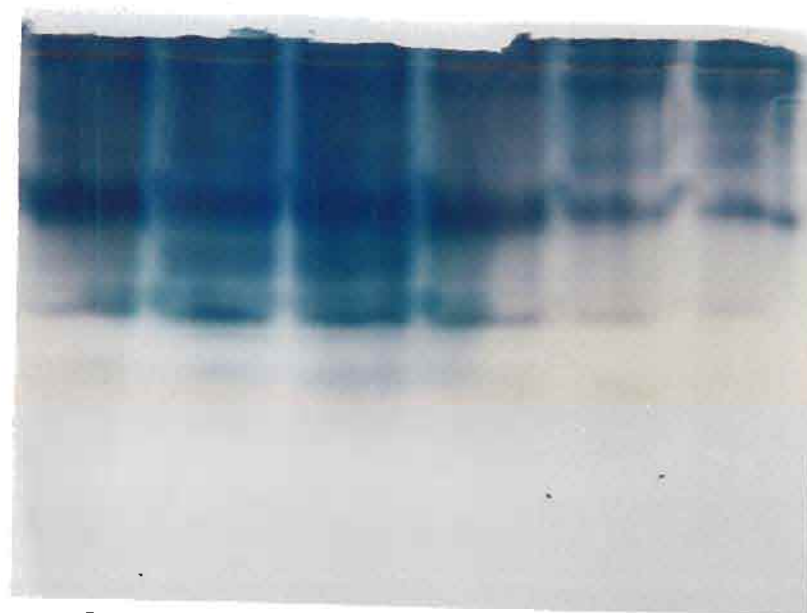
CO 1



Soybean - Leaf

CO 1

CO 2

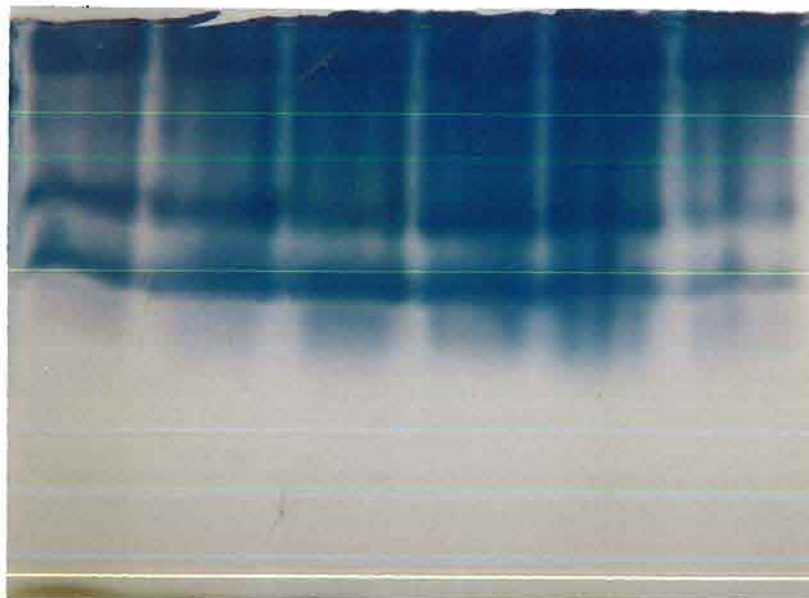


C SDF 10DF C SDF 10DF

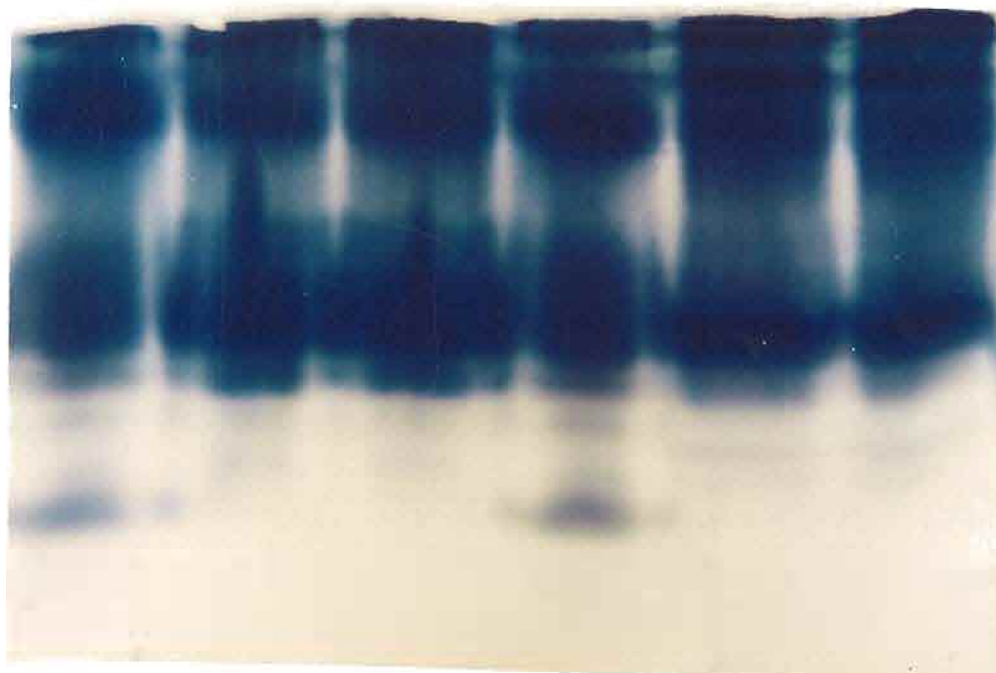
Green gram - Root

CO 4

VBN 1



Green gram - Leaf



C

5DF

10DF

C

5DF

10DF