INFLUENCE OF PUTRESCINE AND INDOLE-3-BUTYRIC ACID ON GROWTH AND PRODUCTIVITY OF SOYBEAN

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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(PLANT PHYSIOLOGY)

By
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Enrolment Number – HH/304

2015

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled "**INFLUENCE OF PUTRESCINE AND INDOLE-3-BUTYRIC ACID ON GROWTH AND PRODUCTIVITY OF SOYBEAN**" or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur

Date: 14/ 05/2015

(Wagh Yogesh Ashokrao)

Enrolment No. HH/304

CERTIFICATE

This is to certify that thesis entitled "**INFLUENCE OF PUTRESCINE AND INDOLE-3-BUTYRIC ACID ON GROWTH AND PRODUCTIVITY OF SOYBEAN"** submitted in partial fulfilment of the requirement for the degree of **"Master of Science in Agriculture** (**Agricultural Botany**)" of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **Wagh Yogesh Ashokrao** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place: Nagpur
Date :14/05/2015

(Dr. R. D. Deotale)
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Countersigned

Associate Dean,

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Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINER (AFTER VIVA -VOCE)

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Place : Nagpur

Date :14/05/2015

(Wagh Yogesh Ahokrao)

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(A) List of Tables

(B) List of Figures

No.	:	Number
log_e	:	Natural logarithm
ppm	:	Parts per million
PCA	:	Polycaboxylic acid

(C) Abbreviations and Symbols

AR	:	Analytical reagent
&	:	And
@	:	At the rate
Cm	:	Centimeter
$CuSO_4$	:	Copper sulphate
CD	:	Critical difference
cv.	:	Cultivar
DAS	:	Days after sowing
dm^2	:	Decimeter square
=	:	Equal to
Etc	:	Etcetera
et al.	:	etalia (and others)
g	:	Gram
ha	:	Hectare
kg	:	Kilogram
L	:	Litre
l^{-1}	:	Per litre
M.S.	:	Maharashtra State
Max.	:	Maximum
MT	:	Metric tones
M	:	Meter
Mg	:	Milligram
Mm	:	Millimeter
N	:	Nitrogen
No.	:	Number
log_e	:	Natural logarithm
ppm	:	Parts per million
PCA	:	Polycaboxylic acid
%	:	Per cent
g^{-1}	:	Per gram

P	:	Phosphorus
K	:	Potassium
KCL	:	Potassium chloride
KOH	:	Potassium hydroxide
$Plant^{-1}$	:	Per plant
$Plot^{-1}$	:	Per plot
Day^{-1}	:	Per day
kg^{-1}	:	Per kilogram
ha^{-1}	:	Per hectare
Q	:	Quintal
RBD	:	Randomized block design
Sp.	:	Species
H_2SO_4	:	Sulphuric acid
Na_2SO_3	:	Sodium sulphite
NaOH	:	Sodium hydroxide
T	:	Treatment
Put	:	Putrescine
IAA	:	Indole acetic acid
IBA	:	Indole-3-butyric acid
PAs	:	Polyamines
LRF	:	Lateral root formation
Var	:	Variety
RDF	:	Recommended dose of fertilizers
µ g	:	Microgram
µ l	:	Micro litre

(D) THESIS ABSTRACT

a)	Title of the thesis	:	**"INFLUENCE OF PUTRESCINE AND INDOLE-3-BUTYRIC ACID ON GROWTH AND PRODUCTIVITY OF SOYBEAN "**
b)	Full name of student	:	**WAGH YOGESH ASHOKRAO**
c)	Name and address of Major Advisor	:	**Dr. R. D. Deotale** Professor of Botany, College of Agriculture, Nagpur
d)	Degree to be awarded	:	M.Sc. (Agri.)
e)	Year of award of degree	:	2015
f)	Major subject	:	Agricultural Botany, (Plant Physiology)
g)	Total number of pages in the thesis	:	63
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i)	Signature of student	:	
j)	Signature, Name and address of forwarding authority	:	

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ABSTRACT

The influence of putrescine (50, 75, 100, 125, 150, ppm) and IBA (50, 75, 100, 125, 150, ppm) on the morpho-physiological, chemical and biochemical, yield and yield contributing parameters of soybean cv. JS-335 was studied during *kharif* 2014-2015 at farm of Botany section, college of Agriculture, Nagpur. Experiment was conducted in RBD with three replications and eleven treatments. Sowing was done by dibbling method on dated 14 July 2014. Gap filling was done and plant protection measures were applied as an when required.

Morpho-physiological parameters such as plant height, number of primary branches, leaf area and dry weight of plant at 30, 45, 60 and 75 DAS were recorded. RGR, NAR were calculated at 30-45, 45-60 and 60-755 DAS. Chemical and biochemical parameters like leaf chlorophyll, NPK content in leaves, protein and oil content in seed were also estimated. Observations on yield and yield contributing parameters like 100 seed weight, number of the pods plant^{-1}, seed yield plant^{-1}, plot^{-1} and ha^{-1} were recorded. All these above mentioned parameters were analyzed statistically.

Two foliar sprays of putrescine (50, 75, 100, 125, 150, ppm) and IBA (50, 75, 100, 125, 150, ppm) significantly enhanced morpho-physiological parameters viz., plant height, number of branches, leaf area, dry weight of plant, RGR, NAR, chemical and biochemical parameters viz., nitrogen, phosphorus, potassium, chlorophyll in leaf, protein and oil content in seed and yield and yield contributing parameters viz., 100 seed weight, number of the pods plant^{-1}, seed yield plant^{-1}, plot^{-1} and ha^{-1} over control.

Considering the treatments under study two foliar sprays of 100 ppm putrescine and 100 ppm IBA at two stages i.e. before flowering and 10 DAS after flowering were found to be most effective in improving morpho-physiological, chemical and biochemical, yield and

yield contributing parameters of soybean cv. JS-335 and ultimately increased yield by 25.89 and 20.34 per cent respectively over control and can be considered as the most effective and beneficial treatment in soybean.

Chapter I

INTRODUCTION

Background information

Soybean (*Glycine max* L.) is a diploid species having chromosome number 2n=40. It belongs to family "Leguminoseae" and subfamily "Papilionoidae". It is annual leguminous herbaceous plant. Soybean is also known as "Gold of Soil" due to its various qualities such as ease in cultivation, less requirement of fertilizers and labour resulting in high cost: benefit ratio. Soybean being a legume crop is gifted naturally to fix atmospheric nitrogen in the root nodules with the help of *Rhizobium* (Anonymous, 1985).

Soybean is one of the important oilseed as well as leguminous crop. It is gaining importance in India and other developing countries to ward off malnutrition. Due to its high nutritive value soybean cultivation has taken great strides during the recent years. It is the cheapest and richest source of high quality protein. It supplies most of the nutritional constituents essential for human health. Hence, soybean is called as "Wonder crop" or "Golden bean" or "Miracle bean". This crop in fact has made revolution in the agricultural economy with its immense potential, quality as food, feed, numerous industrial product commodity. It is soil erosion resistant crop and suited for most of the soil.

Among, oilseeds soybean ranks fifth in the world. The important soybean growing countries in world are America, Brazil, Argentina, China and India. The largest soybean producing state in India are Madhya Pradesh, Maharashtra and Rajasthan. In India Maharashtra having second rank in production.

Table 1. Area, production and productivity of soybean crop during year 2013-2014

Region	Year	Area ("00"ha)	Production ("00" tonnes)	Productivity (kg ha^{-1})
India (a)	2012-2013	120.327	126.832	1079
Maharashtra (b)	2012-2013	38.704	48.565	1255
Vidarbha (c)	2012-2013	5.52	6.75	1223

(Source: Anonymous, a, b, c, 2014)

Importance of study

Soybean the wonder crop as it is called as a boon of malnutrished world. Soybean protein contents all the essential amino acids vital for human diet. Besides protein and oil, soybean contains 20.9 % carbohydrate, 60 % polyunsaturated fatty acids (52.3 % linolenic acid + 7.29 % linoeic acid), Vitamin A, VitaminB, Vitamin C, D, E, K, 0.69 % phosphorus, 0.0115 % iron, 0.0024 % calcium and all the essential amino acids. Amongst oil seed crops it has highest content of lysine (5 %), a limiting factor in cereals. So it is called "Poor mans meal" it's a really true.

The soyfeeds are beneficial to the diabetics and for the patients suffering from rheumatism and high blood cholesterol. Soymilk is a good source of lactose for children. Thus, it may be said that a soymilk drink a day might keep the doctor away. Because of its high nutritive value soybean food like soymilk, curd, soydal, sauce, beverages as well as roasted soybean have gained popularity in many parts of the world. It has high calorific value releasing 395 calorie along with 227 mg calcium, 546 mg phosphorus, 8.8 mg iron, 660 IU vitamin B-1 from100 g edible soybean.

Soybean is used in the production of soymilk, curd, various soyasources, fermented products and bean sprouts. Soya oil used as a raw material for the production of top-quality dye, varnishes, soaps, plastics, strong glues and adhesives. The oil cake contains 44-50 %

protein and is used extensively as a supplement to cereal grains in livestock and poultry feeds. Considering the importance of soybean from nutritional and production point of view, it becomes necessary to cultivate soybean crop with expectation of higher yield. The crop productivity can be increased through physiological approaches by co-ordinating plant process to synthesize dry matter and partitioning its major quantum of effective yield contributing factors. The yield of soybean may be enhanced through physiological manipulation such as foliar application of putrescine and IBA.

Growth mainly refers to quantitative increase in the plant body whereas the qualitative changes refer to development. According to Krishnamoorthy (1981) growth and development of the plant are controlled by internal factors, namely nutritional and hormonal. Nutrional factors supply the plants necessary minerals. They constituent the raw materials required for growth. However, utilization of these substances for a balanced development of the plant body is controlled by certain growth regulators.

Plant growth regulators are substances when added in small amounts modify the growth of plant usually by stimulating or inhibiting part of the natural growth regulation. They are considered as new generation of agrochemicals after fertilizers, pesticides and herbicides. Plant growth regulators are capable of increasing yield by 100-200 per cent under laboratory conditions, 10 -15 per cent in the field conditions. Plant growth regulators like promoters, inhibitors or retardants play a key role in internal control mechanism of plant growth by interacting with key metabolic processes such as nucleic acid and protein synthesis. The most commonly used growth regulators in soybean are IBA, putrescine, Ethrel, cycocel, salicyclic acid, IAA, GA_3 etc. are enhancing growth and productivity of crop plants.

Plant growth regulators are shown to change leaf resistance by altering stomatal aperture, the rate of photosynthesis could be manipulated through this technology. Putrescine, IBA, IAA, GA, kinetin, phenolics and aliphatic alcohols are reported to increase and stimulate the rate of photosynthesis. The diamine putrescine occurred widely in the higher plants. It was suggested to be involved in a variety of growth and developmental processes such as cell division (Bueno and Matilla,1992),fruit set and growth (Biasi *et al.*,1991) and senescence (Kao,1994).

IBA is a plant growth regulator, used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock. IBA is also used on fruit and vegetable crops, field crops and ornamental turf to promote growth development of flowers and fruit and to increase crop yields. IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally-occurring plant growth hormone indole-3-acetic acid.

Application of growth promoting hormones is a recent technique in this direction. Plant hormones in a broad sense are organic compounds which play an important role in plant growth development and yield of crops to prevent the fruit and flower drop for a longer period.

Objectives of study

1. To study the effect of foliar sprays of putrescine and indole-3-butyric acid on morpho-physiological, yield and it's contributing parameters.

2. To study the effect of foliar sprays of putrescine and indole-3-butyric acid on biochemical parameters.

Scope and Limitations

Scope

Putrescine and IBA is also readily available so there is no problem of getting it. If this experiment is successful and if hypothesis is proved then this can be profitably used by the farmer. Foliar spray of

Putrescine and IBA is beneficial for improving growth of the plant. These sources are readily available.

Limitations

1) Foliar application followed by immediate rains within six hours may nullify the effect.

2) Preparation of solutions from putrescine and IBA (ppm) requires knowledge. Hence, it can be difficult for farmers to prepare required concentrations of putrescine and IBA.

Hypothesis

The information generated from the experiment will be useful for recommendation of foliar sprays of putrescine and IBA for improving morpho-physiological, chemical and biochemical parameters leading to increase yield in soybean.

Chapter II

REVIEW OF LITERATURE

Plant growth regulators so far have emerged as "magic chemicals" that could increase agricultural production at an unprecedented rate and help in removing and circumventing many of barries imposed by genetics and environment. Plant growth regulators when added in small amounts, modify the natural growth regulatory system right from seed germination to senescence in several crop plants.

Plant growth regulators play key role in contributing internal mechanisms of plant growth by interacting with key metabolic processes such as, nucleic acid metabolism and protein synthesis.

Plant growth regulators are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and enhance the effective partitioning of accumulates from source and sink in the field crops (Solamani *et al.*, 2001).

Putrescine: Putrescine oxidase (EC 1.4.3.4), putrescine: oxygen oxidoreductase (deaminating) (flavin containing), has been found to form complexes with a variety of amines. With few exceptions these compounds competitively inhibit putrescine oxidation and also perturb the visible absorption spectrum of the enzyme (i.e., the spectrum due to FAD). Inhibition constants are reported for a number of amines; the presence of a cationic amino group in the inhibitors appears to be the structural feature essential for competitive inhibition. Inhibition constants for amino acids are larger than those for the analogous simple amines and the inhibition constants for alkyl mono- and diamines in a homologous series are inversely related to the length of the hydrocarbon chain. Amines containing unsaturated and aromatic substituents yield relatively low inhibition constants. The spectral changes observed upon complex formation are interpreted as indicating a less polar environment for FAD in the enzyme-inhibitor complex than in the uncomplexed enzyme. On the basis of the enzyme's substrate specificity and comparisons among inhibitor structures and the corresponding inhibition constants, a schematic model of the enzyme's active site is proposed,(Swain and Desa 1976).

Mode of action of putrescine

The diamine putrescine occurred widely in the higher plants.It was suggested to be involved in a variety of growth and developmental processes such as cell division (Bueno and Matilla,1992),fruit set and growth (Biasi *et al.*,1991) and senescence (Kao,1994).The interaction of polyamines with the macromolecules was responsible for physiological effects on plant growth and development (Smith,1985).Polyamines were effective on inhibiting RNase and protease activities(Kaur-Sawnney and Galston,1982). Also, Serafmi-Fracassini (1991) found that polyamines are activators of protein process that was related to genes encoding (Imai *et al.*, 2004).

Figure 1- Structure of Putrescine

Plant growth regulators (PGRs) are representing one of the controlling factors that regulate growth, biosynthesis of chemical constituents, yield and may be improve adaptation of plants to environment. Polyamines (PAs) are polycationic compounds of low molecular weight that are present in all living organisms (Cohen, 1998). Some studies have shown the positive effects of PAs on cell division and its rate (Cvikrova *et al.*, 1999), morphogenesis, floral initiation and development (Khan *et al.*, 2012). PAs effectively retard senescence by retarding the loss of chlorophyll (Couee *et al.*, 2004;). Putrescine application (up to 5 mM) significantly increased the content of photosynthetic pigments of wheat (*Triticum aestivum*) plants (El-Bassiouny *et al.*, 2008). PAs have been shown to be an integral part's response to stress (Alcazar *et al.*, 2006; 2010). Exogenous putrescine and sermidine markedly modified the stress-induced effects in plants (Amooaghaie, 2011). Moreover, putrescine treatments significantly increased fresh and dry weights of bean plants. Putrescine at 10^{-5} M increased grain and biological yield and grain index of wheat plant (Gupta *et al.*, 2003). Exogenous application of putrescine and spermidine increased endogenous PGRs, particularly growth promoters (Indole-3- acetic acid, gibberellins and cytokinins) and decreased inhibitors (abscisic acid) (El-Bassiouny and Bakheta, 2005; Iqbal *et al.*, 2006). Moreover, auxins are implicated in various metabolic processes as nucleic acid synthesis (Krishnamoorthy 1981; Alvi *et al.*, 2005).

INDOLE-3-BUTYRIC ACID

IBA is a plant growth regulator, used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock. IBA is also used on fruit and vegetable crops, field crops and ornamental turf to promote growth development of flowers and fruit and to increase crop yields. IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally-occurring plant growth hormone indole-3-acetic acid.

Mode of action of IBA (Indole-3-butyric acid)

In auxin bioassays, IBA often shows only weak activity but there are exceptions. IBA, but not IAA, can efficiently induce adventitious roots in Arabidopsis. Moreover, it has emerged that IBA can promote lateral root formation (LRF) independently of IAA, Altogether, several pieces of evidence indicate that the straight forward scenario of IBA being only a precursor of the active auxin IAA is not sufficient to explain its activity features. It has been shown that IBA, much like IAA, uses NO as a downstream signal to induce lateral roots. NO signal transduction cascades relevant for root primordial formation include cyclic GMP, phospholipase D, phosphatidic acid, ROS and PIN transporters of auxin. Here, we show the effects of IAA and IBA in different root development model systems using wild-type and mutant plant lines of maize and Arabidopsis. Particular emphasis is paid to NO-mediated IAA and IBA effects on root growth and development of root systems. We use the protein degradation inhibitor Terfestatin A as an additional experimental tool. This inhibitor prevents the auxin-specific changes of gene repression and enables discrimination between transcriptional and redox-based activities of auxin

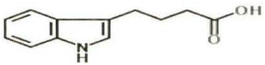


Figure 2- Structure of IBA

Indole-3-butyric acid significantly increased the vegetative growth criteria, seed yield and protein content in rice, maize and onion plants. Soaking chickpea seeds with 10^{-8} M IAA improved nitrate reductase activity, total protein, calcium and potassium contents. Moreover, improvement in nitrogen metabolism, photosynthesis and yield of chickpea were noted with the application of 10^{-8} M of IAA, IBA, or 4-Cl-IAA (4-chloroindole-3-acetic acid).

Effect of putrescine and IBA on morpho-physiological characters

Muthukumar *et al.* (2005) carried out an experiment to study the effect of different plant growth regulators and spit application of nitrogen on the productivity of baby corn. The trial was laid out in split plot design and replicated thrice. Growth regulators viz., Mepiquat chloride @ 200 ppm, NAA @ 40 ppm, Putrescine @ 50 ppm along with water spray (control) were taken in main plots and split application of nitrogen viz., ½ basal + ½ N at 25 DAS, ½ basal + ½ at 45 DAS, ½ basal + ¼ at 25 DAS + ¼ at 45 DAS; and ¼ basal + ½ at 25 DAS + ¼ at 45 DAS were taken in sub plots. The results revealed that growth regulators application had significant influence on growth parameters. (plant height, leaf area index and dry matter production) when compared with control.

Thavaprakash *et al.* (2006) conducted the field experiment on effect of plant growth promoters on growth parameters, assimilate partitioning and seed yield of green gram. The results revealed that foliar application of putrescine and spermine @ 20 ppm registered higher growth characters viz., plant height, number of trifoliate leaves plant^{-1}, leaf area index and dry matter production.

Mathur and Vyas (2007) conducted a field experiment to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Result showed that application of salicylic acid @ 3 mM and sitosterol or putrescine @ 0.15 mM significantly increased vegetative growth characters such as plant height, leaf area, leaf area index, dry weight plant^{-1}, specific leaf weight and net assimilation rate.

Amin *et al.* (2007) tested different concentrations of indole-3-butyric acid (25, 50, and 100 mg l^{-1}) and salicylic acid (50, 100 and 200 mg l^{-1}) and reported that foliar application of indole-3- butyric acid and salicylic acid at 100 mg l^{-1} significantly enhanced plant height, number of leaves plant^{-1}, fresh and dry weight plant^{-1} and leaf area plant^{-1} of onion.

El-Bassiouny *et al.* (2008) tested arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and reported that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased all growth parameters *i.e.* shoot length, number of tillers plant^{-1}, number of leaves plant^{-1}, leaf area, circumference of main stem, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root when applied at 30 or 60 DAS over control.

Shraiy and Hegazi (2009) studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA @ 25 and 35 DAS significantly enhanced plant growth viz., plant height, number of leaves, fresh and dry weight.

Amin *et al.* (2011) tested different concentrations of putrescine (25, 50 and 100 ppm) and glutamine (50, 100 and 200 ppm) either alone or in combination and reported that foliar application of 100 ppm putrescine and 200 ppm glutamine either alone or in combination significantly increased plant height, number of leaves, fresh weight of leaves, fresh and dry weight of plant and leaf area of onion.

Ghodrat *et al.* (2012) studied the effect of indole-3-butyric acid (0, 10 and 20 mg l^{-1}) on wheat. Result showed that indole-3-butyric acid @ 10 mg l^{-1} significantly increased root length and shoot length.

Amin *et al.* (2013) revealed the response of chickpea (*Cicer arientinum* L.) to treatment with two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased the plant height, number of branches, dry weight of leaves and leaf area plant^{-1} over control.

Bideshki *et al.* (2013) conducted a field experiment to study the impact of 0 and 100 ppm indole-3-butyric acid (IBA) and 0, 0.1 and 0.5mM salicylic acid (SA) on garlic and reported that 0.5 mM salicylic acid and 100 ppm IBA significantly enhanced leaf area, root number and plant fresh weight.

Ahmed *et al.* (2013) carried out a field experiment to determine the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly increased plant height, number of leaves plant^{-1}, leaf area plant^{-1}, fruiting branches plant^{-1}, shoot fresh and dry weight.

Effect of putrescine and IBA on biochemical parameters

Mathur and Vyas (2007) conducted an experiment to study the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Result showed that application of salicylic acid at 3 mM and sitosterol or putrescine at 0.15 mM significantly increased crude protein, total carotenoids, total sugar and oil content.

Amin *et al.* (2007) tried different concentrations of indole-3-butyric acid (25, 50, and 100 mg l^{-1}) and salicylic acid (50, 100 and 200 mg l^{-1}) and observed that foliar application of indole-3- butyric acid and salicylic acid @ 100 mg l^{-1} significantly increased photosynthetic pigments, total soluble sugars, total free amino acids, total phenols and total indoles of onion.

El-Bassiouny *et al.* (2008) tried arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and found that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased photosynthetic pigment, carbohydrates, nucleic acid (DNA and RNA), nitrogenous constituents and mineral contents (K, Ca, Mg and p) when applied at 30 or 60 DAS over control.

Shraiy and Hegazi (2009) conducted an experiment to study the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased total chlorophyll in leaves, total soluble proteins, proline, phenol, total soluble carbohydrates and sugar in seeds.

Amin *et al.* (2011) reported that foliar application of 100 ppm putrescine and 200 ppm glutamine either alone or in combination significantly enhanced total soluble sugars, sulphur compounds, total soluble phenols, total free amino acids, and total photosynthetic pigment content in onion leaves.

Amin *et al.* (2013) tester two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly enhanced nitrogen, phosphorus, potassium, total soluble sugars and total free amino acids of chickpea (*Cicer arientinum* L.)

Bideshki *et al.* (2013) carried out a field trial to study the impact of 0 and 100 ppm indole-3-butyric acid (IBA) and 0, 0.1 and 0.5mM salicylic acid (SA) on garlic and reported that 0.5 mM salicylic acid and 100 ppm indole-3-butyric acid significantly increased carotenoid, chlorophyll and allicin contents.

Ahmed *et al.* (2013) conducted a field experiment to study the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly enhanced inorganic N, P and K or organic constituents proline, total free amino acids, total sugars, total soluble phenols, chlorophyll and total carotenoids.

Effect of putrescine and IBA on yield and yield contributing parameters

Muthukumar *et al.* (2005) studied the effect of different plant growth regulators and spit application of nitrogen on the productivity of baby corn. The trial was laid out in split plot design and replicated thrice. Growth regulators viz., mepiquat chloride @ 200 ppm, NAA @ 40 ppm putrescine @ 50 ppm along with water spray (control) were taken in main plots and split application of nitrogen viz., ½ basal + ½ N at 25 DAS, ½ basal + ½ at 45 DAS, ½ basal + ¼ at 25 DAS + ¼ at 45 DAS; and ¼ basal + ½ at 25 DAS + ¼ at 45 DAS were taken in sub plots. The results revealed that growth regulators application significantly increased yield parameters viz., length of cob, diameter of cob, weight of cob, length of corn, diameter of corn, weight of corn, number of cobs, green cob yield and green fodder yield.

Thavaprakash *et al.* (2006) conducted an experiment to study the effect of plant growth promoters on assimilate partitioning and seed yield of green gram. The results revealed that foliar application of putrescine and spermine @ 20 ppm registered significant increase in yield attributing characters such as numbers of flowers plant^{-1}, number of pods plant^{-1}, fertility coefficient, number of filled seeds pod^{-1} and per cent filled seeds over control.

Amin *et al.* (2007) tested different concentrations of indole-3-butyric acid (25, 50, and 100 mg l^{-1}) and salicylic acid (50, 100 and 200 mg l^{-1}) and reported that foliar application of indole-3- butyric acid and salicylic acid @ 100 mg l^{-1} significantly increased bulb length, bulb diameter, bulb weight as well as yield of onion and its quality.

Field experipent was conducted by Mathur and Vyas (2007) to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Result showed that application of salicylic acid @ 3 mM and sitosterol or putrescine @ 0.15 mM significantly increased yield and its components *i.e.* ear length, ear diameter, grain yield plant^{-1}, crop index and 100- grain weight over control.

El-Bassiouny *et al.* (2008) tested arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and reported that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased spike number plant^{-1}, grain number plant^{-1}, 1000-grains weight, straw yield and harvest index when applied at 30 or 60 DAS over control.

Sawan (2008) carried out a field experiment to investigate the influence of nitrogen rates (72,144 or 216 kg N ha^{-1}), phosphorus rates (36 or 72 kg P_2O_5 ha^{-1}) and three growth regulators *i.e.* indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or 1 naphthalen acetic acid (NAA), each was sprayed with 10 ppm at three times i.e. 70, 85 and 100 DAS on cotton. Results showed that IAA, IBA and NAA concentration @ 10 ppm significantly increased number of bolls plant^{-1}, boll weight, seed index, seed cotton yield plant^{-1} and seed cotton and lint yield ha^{-1} over control.

Shraiy and Hegazi (2009) studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased 1000 seeds weight and pod parameters (length of pod, pods number plant^{-1}, seeds number pod^{-1}, pod fresh and dry weight) over control.

Amin *et al.* (2011) observed that foliar application of 100 ppm putrescine and 200 ppm glutamine either alone or in combination significantly increased bulb length, bulb diameter and weight as well as yield of onion and quality of bulb also.

Ghodrat *et al.* (2012) conducted an experiment to study the effect of foliar spray of indole butyric acid (IBA) and gibberellic acid (GA_3) on yield and yield components of corn (*Zea mays* L.) cv. single cross 704. Split plot design was used, where foliar application of two growth regulators GA_3 and IBA during two physiological sensitive growth phases, i.e. 4 to 6 leaf and flowering stages constituted the main plots and concentrations of 0, 50 and 100 mg l^{-1} of both IBA and GA_3 formed the subplots. Evaluated physiological traits include ear length, kernel number area^{-1}, kernel weight, grains row^{-1} and grain yield. In comparison with control, application of IBA and GA_3 during flowering stage increased ear length and grain yield to 26.3 cm and 17.2 ton ha^{-1}, respectively. On the other hand, the interaction between IBA and GA_3 mostly affected the kernel number $area^{-1}$ and number of grains row^{-1}. Additionally, the most effective treatment to increase kernel weight was the application of IBA @ 100 ppm in both growth stages. Generally, the results showed that in both growth stages, application of IBA and GA_3 increased yield and yield components of corn plants.

Amin *et al.* (2013) studied the effect of two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased number of pods, seed yield, straw and biological yield $feed^{-1}$ of chickpea (*Cicer arientinum*)

Bideshki *et al.* (2013) conducted a field experiment to study the effect of 0 and 100 ppm indole-3-butyric acid (IBA) and 0, 0.1 and 0.5mM salicylic acid (SA) on garlic and reported that 0.5 mM salicylic acid and 100 ppm IBA significantly enhanced bulb yield and allicin yield over control.

Ahmed *et al.* (2013) studied the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly increased number of total open and closed bolls, seed cotton yield $plant^{-1}$. Lint percentage and seed index were also increased.

Chapter III

MATERIAL AND METHODS

The project entitled "Influence of putrescine and indole-3-butyric acid on growth and productivity of soybean" was carried out during *kharif* - 2014. The details of the materials used and method adopted for these studies are described in this chapter under following heads.

3.1 Material required

3.1.1 Inputs used for research work

1. Seeds of soybean variety JS-335 were used.

2. Putrescine and IBA at different concentrations were used for spraying at different stages of growth to study its effect.

3. For estimation of chlorophyll, nitrogen, phosphorus, potassium and protein content standard chemicals and reagents were used in this experiment.

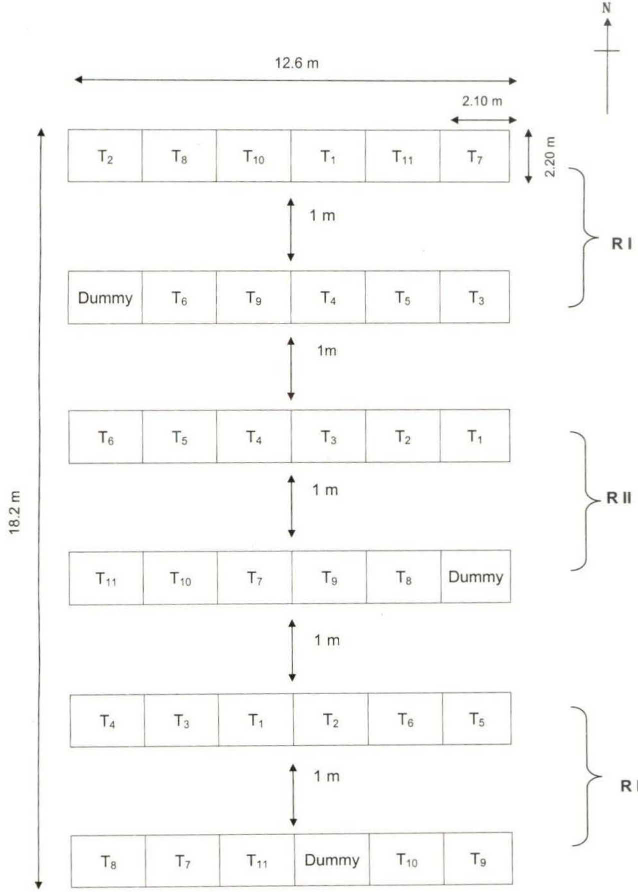
3.1.2. Equipments used

Equipments like electronics balance, leaf area meter, photocell colorimeter, flamephotometer, spectrophotometer, microkjeldhal´s flask, digestion chamber, microkjeldhal´s distillation apparatus etc. were used during this experiment.

3.2. Methods adopted

The field experiment was laid out in Randomized Block Design (RBD) with three replications consisting of eleven treatments comprising of different doses of putrescine and IBA (details in table below).

Seeds were sown at the rate of 75 kg ha^{-1} by dibbling method at a spacing of 30 cm x 10 cm on 14 July 2014 after receiving the sufficient rainfall. After the germination, gap filling was completed on 25



Plot size: Gross plot: 2.10 m X 2.20 m

Net plot: 1.5m X 2.00 m

Figure 3. Layout of experimental field

July 2014. Thinning was carried out after full emergence so as to maintain required number of plants plot^{-1}. Intercultural operations were also undertaken as and when required. Spraying of putrescine and IBA was done two times at 30 and 45 DAS with hand sprayer. Observations were recorded at different stages i.e. at 30, 45, 60 and 75 DAS. The crop was kept free from disease and pest during the growth period. Harvesting in all treatments was undertaken after crop was matured.

3.2.1. Research design and layout

Table 3. Research design

Experimental design	:	RBD (Randomized Block Design)
No. of replications	:	Three (3)
Treatment details	:	Eleven (11)
T_1	:	Control (Water spray)
T_2	:	50 ppm putrescine
T_3	:	75 ppm putrescine
T_4	:	100 ppm putrescine
T_5	:	125 ppm putrescine
T_6	:	150 ppm putrescine
T_7	:	50 ppm IBA
T_8	:	75 ppm IBA
T_9	:	100 ppm IBA
T_{10}	:	125 ppm IBA
T_{11}	:	150 ppm IBA
Plot size	:	Gross plot – 2.10 m x 2.20 m
		Net plot – 1.5 m x 2.00 m
Spacing	:	30 cm x 10 cm
Stages of observations	:	30, 45, 60 and 75 DAS
Cultivar	:	JS-335
Spraying	:	Before flowering (30DAS) and 10 days after flowering (45DAS)
Fertilizer dose	:	30:75:00 kg NPK ha^{-1}

3.2.2 Weather report

Data on weather conditions prevalent during the investigation period (*kharif* 2014-15) recorded at Meterological Observatory, College of Agriculture, Nagpur are given in the table 3.

Table 4. Statement showing the Weekly Meteorological Data for the year 2014-15 recorded at observatory, Agriculture college farm, Nagpur

Date		Met Week	Temp ^{0}C		R.H. %		Total Rain fall (mm)	No. of Rainy days
			Max.	Min.	Mor.	Eve.		
04 – 10	Jun 14	23	41.2	30.5	39	23	-	-
11 – 17		24	39.1	26.7	64	44	26.4	2
18 – 24		25	32.8	25.6	66	54	19.4	2
25 – 01	Jul 14	26	37.5	27.9	57	41	7.4	1
02 – 08		27	33.3	24.8	83	61	22.9	3
09 – 15		28	32.8	24.9	82	58	46.2	4
16 – 22		29	31.1	24.6	86	73	68.6	4
23 – 29		30	26.4	23.9	92	87	99.7	6
30 – 05	Aug 14	31	26.4	23.2	92	86	172.3	5
06 – 12		32	27.1	23.7	88	86	59.1	3
13 – 19		33	28.4	24.1	84	70	21.4	2
20 – 26		34	30.2	24.3	84	69	21.6	3
27 – 02	Sept.14	35	30.5	24.6	85	71	19.8	2
03 – 09		36	30.0	23.6	91	80	241.0	5
10 – 16		37	29.9	24.6	85	72	68.4	3
17 – 23		38	30.8	24.0	81	67	70.8	1
24 – 30		39	32.5	23.4	75	55	2.0	0
01 – 07	Oct.14	40	32.1	23.5	82	59	14.8	2
08 – 14		41	33.0	19.2	66	34	0.0	0
15 – 21		42	32.9	17.9	65	37	0.0	0
22 – 28		43	31.5	18.7	62	34	0.0	0
29 – 04	Nov. 14	44	29.1	18.1	69	52	19.6	2
5 -11		45	30.0	15.5	67	34	0.0	0
12 -18		46	29.6	12.6	63	28	0.0	0
19 -25		47	30.2	14.5	58	39	0.0	0

Total rainfall - 1001.4 mm in 50 rainy days (June 2014 to November 2014)

Rain fall

During this year the monsoon commenced from 24th June, 2014 (24th m.w.) and was continued upto third week of September 2014. It was two days in the month of October 2014 (40th m.w.) and two days in the month of November 2014 (44th m.w). From June to November, total rainfall was 1001.4 mm with 50 rainy days, which was comparatively normal.

Temperature

Temperature during Kharif ranged between 26.40C to 41.2oC (Max.) and 12.6°C to 30.5°C (Min.) and was favorable to crop growth and germination.

Humidity

Average humidity was 76.61% (Max.) and 60.04% (Min.) during *kharif* season of 2014-15.

3.3. Observations

The observations on morpho-physiological, growth analysis, biochemical, chemical, yield and yield contributing parameters were recorded periodically as detailed below.

3.3.1.1 Morpho-physiological observations

a) Plant height plant^{-1} (cm)

Height of 5 randomly selected plants from each treatment was measured at maturity from the base of plant to the terminal bud on primary shoot by using meter scale. Average of 5 plants was then worked out and expressed in cm.

b) Number of branches plant^{-1}

The number of branches were recorded on main stem of the five randomly selected plants and mean branches were recorded.

c) Leaf area plant^{-1} (dm^2)

Leaf area was recorded at 30, 45, 60 and 75 DAS from 5 randomly selected plants. Leaves were detached from five plants and leaf area was then measured by using leaf area meter in cm^2 and then converted into dm^2.

d) Dry weight plant^{-1} (g)

Dry matter of plants was determined periodically at 30, 45, 60 and 75 DAS to assess the dry matter accumulation. For this the plants were uprooted from field. Later on these plants were sun dried and then oven dried at 70°c to record a constant weight. Sufficient care was exercised to avoid charring. The dry weight of plants was recorded on electronic balance (g).

3.3.2 Growth analysis

Growth analysis was carried out by computing relative growth rate and net assimilation rate based on dry matter accumulation, leaf area and period of accumulation as per method suggested by Fischer (1971).

a) RGR (Relative growth rate) (g g^{-1} day^{-1})

Blackman (1919) stated that the increase in dry matter of plants is a process of continuous compound interest; where in the increment in any interval adds to the capital for subsequent growth.

The rate of increment is known as relative growth rate. It was computed by using the Fischer' s formula (1971).

$$RGR\left(g\ g^{-1}\,day^{-1}\right) = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1}$$

Where,

W_1 = Initial dry weight (g)

W_2 = Final dry weight (g)

$T_2 - T_1$ = Time interval (days)

Log e = log10 x 2.303

b) NAR (Net assimilation rate) (g dm^{-2} day^{-1})

It is the increase in dry weight of plant unit^{-1} leaf area in unit time. The NAR was calculated by using the formula suggested by Williams (1946) and expressed as g dm^{-2} day^{-1}.

$$NAR(g\,dm^{-2}\,day^{-1}) = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{LogeLA_2 - LogeLA_1}{LA_2 - LA_1}$$

Where,

LA_1 = Leaf area at time T_1

LA_2 = Leaf area at time T_2

W_2 = Final dry weight (g)

W_1 = Initial dry weight (g)

$T_2 - T_1$ = Time interval (days)

3.3.3 Biochemical and chemical observations

a) Total chlorophyll content in leaves (mg g^{-1})

Total chlorophyll content of oven dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982). Optical density of the extract was measured on double photo cell colorimeter by using red filter.

$$\text{Total Chlorophyll (mg g}^{-1}) = \frac{D652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where,

D = Optical density

V = Final volume of 80% acetone in ml

W = Dry weight of sample taken (g)

b) Estimation of Nitrogen, Phosphorus and Potassium in leaves

i) Nitrogen (%)

Nitrogen content in leaves was determined by micro-kjeldhal's method as given by Somichi *et al.* (1972).

$$\text{Nitrogen (\%)} = \frac{\text{Sample titration – blank titration x N. of HCL x 14x100}}{\text{Sample weight x 1000}}$$

ii) Phosphorus (%)

Phosphorus content in leaves was determined by vanadomolybdate yellow colour method as given by Jackson (1967).

$$\text{Phosphorus (\%)} = \frac{X}{10^6} \times \frac{\text{Volume of acid extract finally prepared x Volume made up}}{\text{aliquot taken x weight of sample in (g)}} \times 100$$

Where,

X= graph reading

iii) Potassium (%)

Potassium content in leaves was determined by flame photometer by di-acid extract method given by Jackson (1967).

$$\text{Potassium (\%)} = \frac{X}{10^6} \times \text{vol. of extract (ml)} \times \frac{100}{\text{Sample (g)}} \times \text{DF}$$

Where,

R = Flame photometer reading of sample

F = Factor from std. graph [Reading (ppm)]

DF = Dilution factor

c. Estimation of protein content in seed (%)

Nitrogen content in seed was determined by micro-kjeldhal's method (Somichi *et al.*, 1972) and same was converted in to crude protein by multiplying 'N' percentage with factor 5.76.

Protein content in seed = N% x 5.76

d. Oil content in seed (%)

The seeds obtained from each treatment and replications were used individually for estimation of oil content of soybean cultivars by Soxhlet's procedure described by Sankaran (1965).

$$\text{Oil \%} = \frac{W_2 - W_1}{W_0} \times 100$$

Where,

W_1 = Blank weight of extraction flask (g)

W_2 = Weight of extraction flask containing oil (g)

W_o = Weight of seed taken (g)

3.3.4 Yield and yield contributing parameters

a. Number of pods plant^{-1}

The numbers of pods of five randomly selected plants were counted prior to harvest and mean number of pods plant^{-1} was calculated.

b. 100 seed weight (g)

100 seeds were counted from five randomly selected plants and weighed in gram separately on precision electronic balance and mean 100 seed weight plant^{-1} was recorded.

c. Seed yield plant^{-1} (g)

The grain yield obtained from five randomly selected plants were weighed in gram separately on precision electronic balance and mean grain yield plant^{-1} was recorded.

d. Seed yield plot^{-1}(kg) and ha^{-1}(q)

Each treatment plot was harvested and threshed separately, grain obtained were weighed separately, to record the grain yield plot^{-1} and ha^{-1}.

e. Harvest Index (%)

It is the coefficient of effectiveness. It can be defined as per cent ratio of economic yield to biological yield. It is the real index of dry matter partitioning into economically important produce. It was calculated by the procedure suggested by Donald and Hamblin (1976).

$$\text{Harvest Index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.4 Method and formula

Experimental plot wise data on morpho-physiological, chemical and biochemical and yield and yield contributing parameters were collected and they were subjected to the statistical analysis by employing the method for RBD as suggested by Panse and Sukhatme (1954).

1. Analysis of variance for the experimental design

The analysis of variance was performed to test the significance of differences between the treatment for all the characters as per the methodology suggested by Panse and Sukhatme (1954).

The statistical model used for the design was:

$$Y_{ijk} = \mu + V_{ij} + b_k + e_{ijk}$$

Where,

Y_{ijK} = performance of ij^{th} treatment over k^{th} replication

μ = general mean

V_{ij} = the effect of the ij^{th} treatment

b_k = block effect

e_{ijk} = the environmental effect

The analysis of variance based on this model is given below

Source of variation	d.f.	Mean squares	Expected mean squares
Replications	(r-1)	MSr	$\sigma^2e + t\sigma^2r$
Treatments	(t-1)	MSt	$\sigma^2e + r\sigma^2t$
Error	(t-1)(r-1)	MSe	σ^2e

Where,

r = Number of replications

t = Number of treatments

The standard error of difference for comparing any two progeny means computed by using appropriate error as follows:

$$SSr = \frac{\sum (\text{replications total})^2}{\text{Number of treatments}} - \text{correction factor}$$

$$SSt = \frac{\sum (\text{treatments total})^2}{\text{Number of replications}} - \text{correction factor}$$

SSe = Total SS. - (Rep. S.S. + Treat S.S.)

$$MSr = \frac{SSr}{(r-1)}$$

$$Mst = \frac{SSt}{(t-1)}$$

$$MSe = \frac{SSe}{(r-1)(t-1)}$$

The F- test was applied to determine the significance of all treatments. Standard error and critical difference at 5% level of probability was used for comparing treatment differences.

$$\text{Error mean sum of sqare (EMS)} = \frac{E\,ss}{edf}$$

S.E.(m) = Standard error of treatment means

$$= \sqrt{\frac{EMS}{r}}$$

C.D. = Critical difference = S.E (m) x t (Edf) x $\sqrt{2}$

Where,

edf	=	error degree of freedom
r	=	number of replications
t	=	number of treatments
t(edf)	=	Table value for degrees of freedom at 5%

3.5 Place/ Duration/Season of experiments

The field experiment was conducted at the farm of Botany Section, College of Agriculture, Nagpur (MS) during *kharif* 2014-15.

Chapter IV

RESULTS AND DISCUSSION

The result of morphophysiological, biochemical, yield and yield contributing parametes as influenced by the foliar sprays of different concentrations of putrescine i.e. 50, 75, 100, 125, 150 ppm and IBA i.e. 50, 75, 100, 125, 150 ppm on soybean are presented and interpreted in this chapter.

4.1 Morpho-physiological parameters

4.1.1 Plant height (cm)

Plant height is important and visible measure of plant growth. Plant height is a function of inter node elongation and leaf emergence. Since leaves are born on stem, leaf area development and biomass production shows a close relationship with plant height.

Data regarding plant height were recorded at 30, 45, 60, and 75 DAS. The data are presented in table 4 and illustrated through figure 4.

At 30 DAS the data regarding plant height was found non significant, because foliar sprays of putrescine and IBA were given from this stage onwards (30-45 DAS).

At 45 DAS significantly maximum plant height was recorded in treatment T_4 (100 ppm putrescine) followed by treatment T_9 (100 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_3 (75 ppm putrescine), T_8 (75 ppm IBA), T_5 (125 ppm putrescine) and T_{10} (125 ppm IBA) were found significantly superior over treatment T_1 (control). Treatments T_{11} (150 ppm IBA), T_2 (50 ppm putrescine), T_6 (150 ppm putrescine) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

At 60 DAS significantly maximum plant height was recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100

Table 4. Effect of putrescine and IBA on plant height plant^{-1} (cm) in soybean

Treatments	Plant height (cm)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	17.73	29.73	48.90	53.40
T_2 (50 ppm Putrescine)	17.93	31.10	50.56	54.10
T_3 (75 ppm Putrescine)	17.70	32.46	52.50	57.36
T_4 (100 ppm Putrescine)	17.66	34.76	55.13	58.13
T_5 (125 ppm Putrescine)	18.10	32.26	51.30	56.13
T_6 (150 ppm Putrescine)	18.33	30.06	51.00	55.14
T_7 (50 ppm IBA)	17.23	30.00	49.06	54.03
T_8 (75 ppm IBA)	17.50	32.43	53.40	56.90
T_9 (100 ppm IBA)	17.56	34.06	54.10	57.86
T_{10} (125 ppm IBA)	17.40	32.16	52.30	56.60
T_{11} (150 ppm IBA)	17.43	31.26	51.96	55.46
SE (m) ±	0.406	0.545	1.270	0.619
CD at 5%	-	1.609	3.752	1.830

ppm IBA) and T_8 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_3 (75 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

At 75 DAS significantly maximum plant height was recorded in treatment T_4 (100 ppm putrescine) followed by treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA) and T_{10} (125 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_5 (125 ppm putrescine) and T_{11} (150 ppm IBA) were found significantly superior over treatment T_1 (control). Treatments T_6 (150 ppm putrescine), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

The per cent increase in plant height with respect to foliar application of 100 ppm putrescine were 16.91 at 45 DAS, 12.74 at 60 DAS and 8.85 at 75 DAS when compared with control (T_1).

Putrescine increased the growth character viz., plant height. This increased growth is due to polyamine application implied that they could act as a growth promoters. It also act as a source of nitrogen (Mirza and Bagni, 1991). Increased growth and allied character viz., plant height might be due to increased chlorophyll content (Thavaprakash *et al.*, 2006). Application of polyamines would increase Rubisco activity (Pyke and Leech, 1987) as elevation of sink 'demand' on developing plants or increase Rubisco enzyme concentration in source leaves which resulted in increased growth and growth allied characters. IBA stimulate dry mass production through enhancement of cell division and chlorophyll accumulation and in turn reflected on the increase in vegetative growth (Amin *et al.*, 2007).This might be the reasons for increase in plant height in the present investigation.

The above observations are in consonance with the findings of following scientists.

Amin *et al.* (2011) tested different concentrations of putrescine (25, 50 and 100 ppm) and glutamine (50, 100 and 200 ppm) either alone or in combination and reported that foliar application of 100 ppm putrescine and 200 ppm glutamine either alone or in combination significantly increased plant height of onion.

Amin *et al.* (2013) revealed the response of chickpea (*Cicer arientinum* L.) to treatment with two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased the plant height over control.

4.1.2 Number of branches

More number of branches plant^{-1} is one of the main parameter which contributes to high yield. Since, leaves are born on stems, leaf area development and biomass production shows a close relationship with plant height and number of branches.

The data regarding number of branches plant^{-1} as influenced by foliar spray of putrescine and IBA are presented in table 5 and illustrated through figure 5.

Data regarding number of branches were recorded at 45, 60 and 75 DAS found statistically significant.

At 45 DAS significantly maximum number of branches were recorded in treatment T_4 (100 ppm putrescine) followed by treatment T_9 (100 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments, T_8 (75 ppm IBA), T_3 (75 ppm putrescine) and T_5 (125 ppm putrescine) were found significantly superior over treatment T_1 (control). Treatments T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_2 (50 ppm putrescine), T_7 (50 ppm IBA) and T_{11} (150 ppm IBA) were found at par with treatment T_1 (control) in respect of number of branches plant^{-1}.

Table 5. Effect of putrescine and IBA on number of branches in soybean

Treatments	Number of branches			
	30DAS	45DAS	60 DAS	75 DAS
T_1 (Control)	2.30	2.53	2.86	2.93
T_2 (50 ppm Putrescine)	2.36	2.86	3.00	3.23
T_3 (75 ppm Putrescine)	2.43	3.26	4.26	4.50
T_4 (100 ppm Putrescine)	2.33	4.00	4.66	4.73
T_5 (125 ppm Putrescine)	2.76	3.13	3.26	3.66
T_6 (150 ppm Putrescine)	2.60	3.00	3.13	3.26
T_7 (50 ppm IBA)	2.50	2.73	3.13	3.16
T_8 (75 ppm IBA)	2.66	3.33	4.00	4.33
T_9 (100 ppm IBA)	2.30	3.66	4.33	4.40
T_{10} (125 ppm IBA)	2.40	2.96	3.66	3.66
T_{11} (150 ppm IBA)	2.43	2.63	3.33	3.40
SE (m) ±	0.146	0.187	0.259	0.194
CD at 5%	-	0.554	0.766	0.575

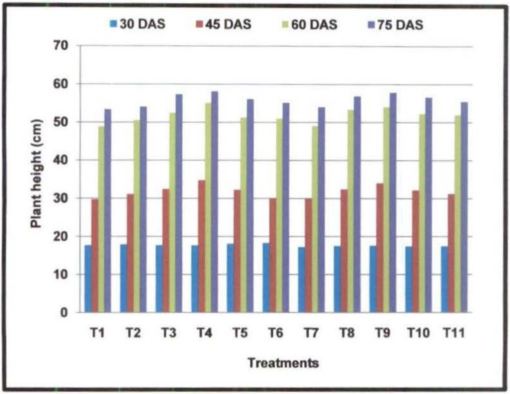


Figure 4. Effect of putrescine and IBA on plant height plant^{-1} (cm) in soybean

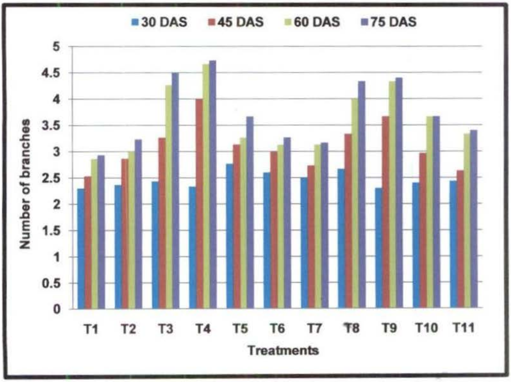


Figure 5. Effect of putrescine and IBA on number of branches in soybean

At 60 DAS significantly maximum number of branches were recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) in a descending manner when compared with T_1 (control) and remaining treatments under study. Treatment T_{10} (125 ppm IBA), also gave significantly more number of primary branches over T_1 (control). But treatments T_{11} (150 ppm IBA), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) were found at par with each other.

At 75 DAS significantly maximum number of branches were recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_3 (75 ppm putrescine), T_9 (100 ppm IBA) and T_8 (75 ppm IBA) in a descending manner when compared with T_1 (control) and remaining treatments under study. Treatments T_5 (125 ppm putrescine) and T_{10} (125 ppm IBA) also gave significantly more number of primary branches over T_1 (control). But treatments T_{11} (150 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were found at par with each other.

Amin *et al.* (2013) revealed the response of chickpea (*Cicer arientinum* L.) to treatment with two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased the number of branches over control.

Ahmed *et al.* (2013) carried out a field experiment to determine the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly increased fruiting branches.

4.1.3 Leaf area of plant

Leaf area play a key role in absorption of radiation in the deposition of photosynthesis during the diurnal and seasonal cycle and in the pathways and rates of bio-geochemical cycling within the canopy soil systems. Leaf area depends on the number and size of the leaves and hence the total leaf area is important parameter for assessing the ability of plants to synthesis its dry matter. The photosynthesis capacity of plants is a function of leaf area development.

Data on leaf area plant^{-1} were recorded at four stages viz., 30, 45, 60 and 75 DAS are furnished in table 6 and figure 6. Leaf area recorded at 45, 60 and 75 DAS gave significant results.

At 30 DAS the data regarding on leaf area was found non significant, because foliar sprays of putrescine and IBA were given from this stage onwards (30-45 DAS).

At 45 DAS significantly maximum leaf area was noticed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. While, treatments, T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_2 (50 ppm putrescine), T_7 (50 ppm IBA) and T_6 (150 ppm putrescine) were found at par with treatment T_1 (control). The range of leaf area at 45 DAS was 4.39 - 7.90 dm^2. The per cent increase in leaf area by treatment T_4 (100 ppm putrescine) over treatment T_1 (control) was 79.95.

At 60 DAS significantly maximum leaf area was recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and rest of the treatments under study. Treatments T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were also produced significantly more leaf area over treatment T_1 (control). The range of leaf area at 60 DAS was 10.13 - 12.51 dm^2. The per cent increase in leaf area by treatment T_4 (100 ppm putrescine) over treatment T_1 (control) was 23.49.

Table 6. Effect of putrescine and IBA on leaf area $plant^{-1}$ (dm^2) in soybean

Treatments	Leaf area (dm^2)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	1.62	4.39	10.13	10.06
T_2 (50 ppm Putrescine)	1.57	4.84	10.76	10.65
T_3 (75 ppm Putrescine)	1.65	6.94	12.04	11.92
T_4 (100 ppm Putrescine)	1.70	7.90	12.51	12.35
T_5 (125 ppm Putrescine)	1.64	5.38	11.23	11.12
T_6 (150 ppm Putrescine)	1.59	4.48	11.07	11.00
T_7 (50 ppm IBA)	1.68	4.76	10.71	10.65
T_8 (75 ppm IBA)	1.64	6.01	11.07	11.00
T_9 (100 ppm IBA)	1.67	7.70	12.09	12.05
T_{10} (125 ppm IBA)	1.58	5.83	11.04	10.94
T_{11} (150 ppm IBA)	1.60	5.06	10.84	10.76
SE (m) ±	0.296	0.665	0.191	0.248
CD at 5%	-	1.964	0.566	0.733

At 75 DAS significantly maximum leaf area was noticed in treatment T_4 (100 ppm putrescine) followed by treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments, T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_6 (150 ppm putrescine) and T_{10} (125 ppm IBA) were found significantly superior over treatment T_1 (control). Treatments T_{11} (150 ppm IBA), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) were found at par with treatment T_1 (control). The range of leaf area at 75 DAS was 10.06 - 12.35 dm^2. The per cent increase in leaf area by treatment T_4 (100 ppm putrescine) over treatment T_1 (control) was 22.76.

Data revealed that leaf area increased from 30 to 60 DAS. But at 75 DAS leaf area decreased. It might be due to leaf fall at this stage.

Putrescine increased the growth character viz., leaf area. This increased growth in soybean due to polyamine application implied that they could act as a growth promoter. Putrescine could act as a source of nitrogen (Mirza and Bagni, 1991). At lower concentrations is comparable to that auxin (Mulkey *et al.*, 1982) and ethylene (Estellie and Somervillie, 1987; Gifford and Evans, 1981). It can also induce the synthesis of RNase enzyme, which enhance the 'N' use efficiency (Bagni *et al.*, 1981). Similarly IBA stimulates dry matter production through enhancement of cell division and chlorophyll accumulation and in turn reflects on the increase in vegetative growth (Amin *et al.*, 2007).This might be the reasons for increase in leaf area in the present investigation.

El-Bassiouny *et al.* (2008) tested arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and reported that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased leaf area when applied at 30 or 60 DAS over control.

Bideshki *et al.* (2013) conducted a field experiment to study the impact of 0 and 100 ppm indole-3-butyric acid (IBA) and 0, 0.1 and 0.5mM salicylic acid (SA) on garlic and reported that 0.5 mM salicylic acid and 100 ppm IBA significantly enhanced leaf area.

4.1.4 Dry weight of plant

Total dry matter accumulation is one of the factor that determines economic yield in crop species where the seed is of economic importance. Leaf is the major organ where most of photosynthates are produced. The number of leaves and their arrangement on the main stem and side branches determine the structure of crop canopy which ultimately decides the dry matter production at each growth stage and its partitioning to reproductive organs during pre-flowering to maturity period has immense importance in determining the final productivity.

Data on dry weight plant were recorded at the four growth stages i.e. 30, 45, 60 and 75 DAS are presented in table 7 and figure 7. Data on dry matter showed significant variation at 45, 60 and 75 DAS.

At 30 DAS the data regarding dry matter was found non significant, because foliar sprays of putrescine and IBA at different concentrations were given from this stage onwards (30-45 DAS).

Significant variation with gradual increase (45-60 DAS) was noticed regarding dry matter production at the stages of observations. The data recorded about dry matter production was subjected to statistically significant.

At 45 DAS significantly maximum dry matter was noticed in treatment T_4 (100 ppm putrescine) followed by treatment T_3 (75 ppm putrescine) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_9 (100 ppm IBA), T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_{10} (125 ppm IBA) and T_6 (150 ppm putrescine) were found significantly

Table 7. Effect of putrescine and IBA on dry weight of plant (g) in soybean

Treatments	dry weight of plant (g)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	3.51	4.60	9.22	12.20
T_2 (50 ppm Putrescine)	3.14	5.26	13.20	16.72
T_3 (75 ppm Putrescine)	3.24	9.47	17.30	27.26
T_4 (100 ppm Putrescine)	3.27	10.16	19.95	30.16
T_5 (125 ppm Putrescine)	3.42	8.08	15.28	21.46
T_6 (150 ppm Putrescine)	3.32	6.26	15.07	17.30
T_7 (50 ppm IBA)	3.59	4.72	12.46	15.24
T_8 (75 ppm IBA)	4.02	8.30	17.57	24.06
T_9 (100 ppm IBA)	3.95	9.22	18.38	28.10
T_{10} (125 ppm IBA)	3.51	7.15	16.16	23.03
T_{11} (150 ppm IBA)	4.10	5.20	15.00	19.79
SE (m) ±	0.339	0.298	1.272	1.262
CD at 5%	-	0.882	3.759	3.731

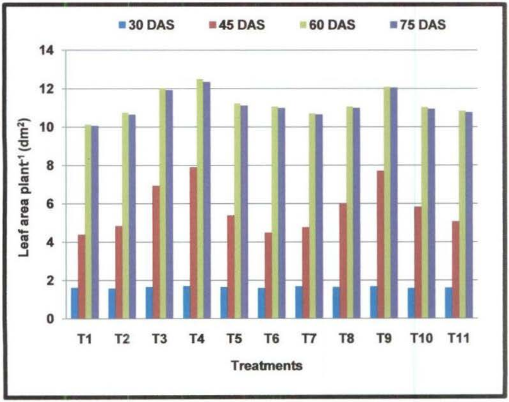


Figure 6. Effect of putrescine and IBA on leaf area plant^{-1} (dm^2) in soybean

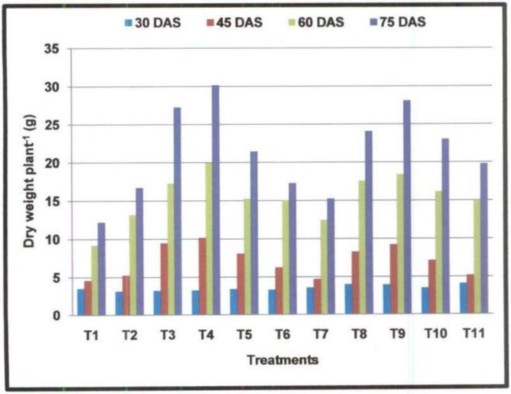


Figure 7. Effect of putrescine and IBA on dry weight of plant (g) in soybean

superior over treatment T_1 (control). Treatments, T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

At 60 DAS significantly maximum dry matter was noticed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_8 (75 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_{11} (150 ppm IBA) and T_2 (50 ppm putrescine) were found significantly superior over treatment T_1 (control). Treatment T_7 (50 ppm IBA) was found at par with treatment T_1 (control).

At 75 DAS significantly maximum dry matter was noticed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA) and T_6 (150 ppm putrescine) were found significantly superior over treatment T_1 (control). Treatments T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) were found at par with treatment T_1 (control).

Significant increase in dry matter from 45-75 DAS might be due to increase in the leaf area and photosynthetic capacity.

Increase in dry matter production by the application of IBA might be due to enhancement of cell division and chlorophyll accumulation (Amin *et al.*, 2007). Similarly putrescine (polyamines) application implied that they could act as a growth promoter (Mirza and Bagni, 1991). This might be the reason for increase in dry weight due to application of putrescine in the present investigation.

Mathur and Vyas (2007) conducted a field experiment to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet

(*Pennisetum typhoides*). Results showed that application of salicylic acid @ 3 mM and sitosterol or putrescine @ 0.15 mM significantly increased dry weight.

Shraiy and Hegazi (2009) studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA @ 25 and 35 DAS significantly enhanced dry weight.

4.2 Growth analysis

The term plant growth analysis refers to useful set of quantitative methods that described and interpret the performance of whole plants systems grown under natural, seminatural, or controlled conditions. Plants growth analysis provides an explanatory, holistic and integrative approach to interpreting plants form and function. It uses simple primary data such as weight, area, volume and content or plant components to investigate processes within and involving the whole plant or crops. The productivity of crop may be related with the parameters such as RGR, NAR and partitioning of total photosynthates into economic and non-economic sink.

The analyses data of RGR and NAR are presented in table 8 and graphically interpreted in figure 8 and 9.

4.2.1 Relative growth rate

Relative growth rate (RGR) represents total dry weight gained over existing dry weight in unit time. This was originally termed an "efficiency index" because it expresses growth in terms of a rate of increase in size $unit^{-1}$ of size. As such, it permits more equitable comparisons between organisms than does absolute growth rate. Normally, relative growth rate deals with total dry weight $plant^{-1}$, though other measures of size have also been used. Data revealed that RGR was more during the period of 45-60 DAS and then 30-45 DAS as compared to RGR at 60-75 DAS.

Considering all the treatments under study, significantly maximum RGR was recorded in treatment with 100 ppm putrescine (T_4) i.e., 0.0472 g $g^{-1}day^{-1}$ at 30-45 DAS, 0.0519 g $g^{-1}day^{-1}$ at 45-60 DAS and 0.0340 g $g^{-1}day^{-1}$ at 60-75 DAS respectively. But it was lowest in control i.e., 0.0413 g $g^{-1}day^{-1}$ at 30-45 DAS, 0.0422 g $g^{-1}day^{-1}$ at 45-60 DAS and 0.0252 g $g^{-1}day^{-1}$ at 60-75 DAS respectively.

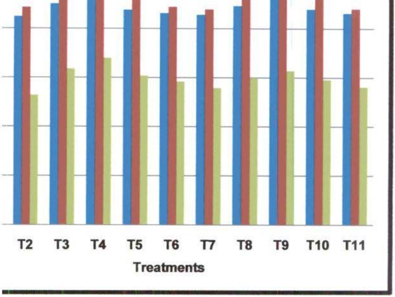
At first stage i.e. 30-45 DAS range of RGR recorded was 0.0413–0.0472 g g^{-1} day^{-1}. Significantly maximum RGR was observed in treatment T_4 (100 ppm putrescine) followed by treatment T_9 (100 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. Also, treatments T_3 (75 ppm putrescine), T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) were found significantly superior over treatment T_1 (control).

At second stage i.e. 45-60 DAS range of RGR recorded was 0.0422-0.0519 g g^{-1} day^{-1}. Significantly maximum RGR was observed in treatment T_4 (100 ppm putrescine). Next to this treatment, treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) also gave maximum RGR when compared with treatment T_1 (control).

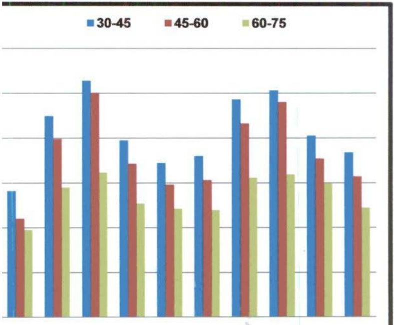
At third stage i.e. 60-75 DAS range of RGR recorded was 0.0252-0.0340 g g^{-1} day^{-1}. Significantly maximum RGR was observed in treatment T_4 (100 ppm putrescine). Next to this treatment, treatments T_3 (75 ppm putrescine), T_9 (100 ppm IBA), T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) also gave maximum RGR when compared with treatment T_1 (control).

Table 8: Effect of putrescine and IBA on RGR (g g^{-1} day^{-1}) and NAR (g dm^{-2} day^{-1}) in soybean

Treatments	RGR (g g^{-1} day^{-1})			NAR (g dm^{-2} day^{-1})		
	30-45	45-60	60-75	30-45	45-60	60-75
T_1	0.0413	0.0422	0.0252	0.0406	0.0338	0.0298
T_2	0.0424	0.0443	0.0264	0.0564	0.0440	0.0390
T_3	0.0450	0.0484	0.0318	0.0898	0.0796	0.0580
T_4	0.0472	0.0519	0.0340	0.1055	0.1002	0.0647
T_5	0.0438	0.0471	0.0304	0.0790	0.0686	0.0508
T_6	0.0431	0.0444	0.0292	0.0689	0.0592	0.0484
T_7	0.0428	0.0439	0.0279	0.0720	0.0612	0.0478
T_8	0.0446	0.0478	0.0300	0.0972	0.0864	0.0623
T_9	0.0466	0.0496	0.0314	0.1012	0.0960	0.0638
T_{10}	0.0439	0.0468	0.0296	0.0810	0.0708	0.0599
T_{11}	0.0431	0.0440	0.0281	0.0736	0.0628	0.0488
SE(m) ±	0.00027	0.00034	0.00035	0.0003	0.00028	0.00026
CD at 5%	0.00078	0.00101	0.00102	0.0009	0.00083	0.00078



f putrescine and IBA on RGR (g g^{-1} day^{-1}) in soybean



4.2.2 Net assimilation rate

NAR is the rate of increasing the dry weight of a plant $unit^{-1}$ of active growing material. NAR is any attribute of the plant which is primarily concerned in carbon assimilation and thus has some claims to be taken as a measure of the internal factor for growth. NAR is closely associated with photosynthesis efficiency of leaves, but it is not a pure measure of photosynthesis. NAR depends upon the excess dry matter gained, over the loss in respiration. It is increase in plants dry weight $unit^{-1}$ area of assimilation tissues $unit^{-1}$ time.

Considering all the treatments under study, significantly maximum NAR was recorded in treatment with 100 ppm putrescine (T_4) i.e. 0.1055 g dm^{-2} day^{-1} at 30-45 DAS, 0.1002 g dm^{-2} day^{-1} at 45-60 DAS and 0.0647 g dm^{-2} day^{-1} at 60-75 DAS respectively. But it was lowest in control i.e. 0.0406 g dm^{-2} day^{-1} at 30-45 DAS, 0.0338 g dm^{-2} day^{-1} at 45-60 DAS and 0.0298 at 60-75 DAS respectively.

At first stage i.e. 30-45 DAS range of NAR recorded was 0.0406-0.1055 g dm^{-2} day^{-1}. Significantly maximum NAR was observed in treatment T_4 (100 ppm putrescine). Next to this treatment, treatments T_9 (100 ppm IBA), T_8 (75 ppm IBA), T_3 (75 ppm putrescine), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA), T_6 (150 ppm putrescine) and T_2 (50 ppm putrescine), also gave maximum NAR when compared with treatment T_1 (control).

At second stage i.e. 45-60 DAS range of NAR recorded was 0.0338-0.1002 g dm^{-2} day^{-1}. Significantly maximum NAR was observed in treatment T_4 (100 ppm putrescine). Next to this treatment, treatments T_9 (100 ppm IBA), T_8 (75 ppm IBA), T_3 (75 ppm putrescine), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA), T_6 (150 ppm putrescine) and T_2 (50 ppm putrescine), also gave maximum NAR when compared with treatment T_1 (control).

At third stage i.e. 60-75 DAS range of NAR recorded was 0.0298-0.0647 g dm^{-2} day^{-1}. Significantly maximum NAR was observed in treatment T_4 (100 ppm putrescine). Next to this treatment, treatments T_9 (100 ppm IBA), T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_3 (75 ppm putrescine), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_6 (150 ppm putrescine), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine), also gave maximum NAR when compared with treatment T_1 (control).

Mathur and Vyas (2007) conducted a field experiment to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Results showed that application of salicylic acid @ 3 mM and sitosterol or putrescine @ 0.15 mM significantly increased net assimilation rate.

4.3 Chemical and biochemical parameters

The chemical and biochemical studies viz., chlorophyll and N, P, K content in leaves as well as oil content in seeds estimated at various stages of observations have been presented here under.

4.3.1 Leaf chlorophyll content

Chlorophyll is a green pigment present in chloroplast of all green plant cells and tissues. These are essential photosynthetic pigments capable of absorbing light energy for the synthesis of carbohydrates. Chlorophyll content of the plant tissue represents the photosynthetic capacity of the plant.

The treatment effects were found statistically significant at 45, 60 and 75 DAS stages of observations except 30 DAS.

Data regarding leaf chlorophyll content in leaves of soybean are presented in table 9 and graphically depicted in figure 10.

Data indicated that, at 45 DAS chlorophyll content was significantly increased in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_8 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study.

Table 9. Effect of putrescine and IBA on leaf chlorophyll content ($mg\ g^{-1}$) in soybean

Treatments	Leaf chlorophyll content ($mg\ g^{-1}$)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	1.31	1.72	2.02	1.99
T_2 (50 ppm Putrescine)	1.39	1.76	2.05	2.03
T_3 (75 ppm Putrescine)	1.28	1.86	2.18	2.16
T_4 (100 ppm Putrescine)	1.45	2.03	2.23	2.21
T_5 (125 ppm Putrescine)	1.43	1.82	2.10	2.08
T_6 (150 ppm Putrescine)	1.44	1.75	2.08	2.06
T_7 (50 ppm IBA)	1.30	1.79	2.08	2.07
T_8 (75 ppm IBA)	1.34	1.92	2.13	2.10
T_9 (100 ppm IBA)	1.32	1.97	2.19	2.17
T_{10} (125 ppm IBA)	1.26	1.83	2.11	2.08
T_{11} (150 ppm IBA)	1.32	1.81	2.09	2.08
SE (m) ±	0.099	0.053	0.040	0.039
CD at 5%	-	0.159	0.121	0.117

Treatments T_3 (75 ppm putrescine), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA), T_2 (50 ppm putrescine) and T_6 (150 ppm putrescine) were found at par with treatment T_1 (control). The range of chlorophyll content at 45 DAS in soybean was 1.72 - 2.03 mg g^{-1}. The per cent increase in chlorophyll content at 45 DAS in treatment T_4 (200 ppm putrescine) over T_1 (control) was 18.02.

At 60 DAS the treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) noted significantly maximum chlorophyll content over treatment T_1 (control) and rest of the treatments under study. But treatments T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_6 (150 ppm putrescine), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) and were found at par with treatment T_1 (control). The range of chlorophyll content at 60 DAS in soybean was 2.02 - 2.23 mg g^{-1}. The per cent increase in chlorophyll content at 60 DAS in treatment T_4 (100 ppm putrescine) over T_1 (control) was 10.39.

At 75 DAS the treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) was most effective in increasing chlorophyll content in leaves when compared with treatment T_1 (control) and rest of the treatments under study. But treatments T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_5 (125 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA), T_6 (150 ppm putrescine) and T_2 (50 ppm putrescine) and were found at par with treatment T_1 (control). The range of chlorophyll content at 75 DAS in soybean was 1.99 - 2.21 mg g^{-1}. The per cent increase in chlorophyll content at 60 DAS in treatment T_4 (100 ppm putrescine) over T_1 (control) was 11.05.

Putrescine or IBA treatments might retard chlorophyll destruction and increase their biosynthesis or stabilize the thylakoid membrane. Polyamines may retard senescence and chlorophyll loss by altering the stability and permeability of membranes and protecting chloroplast from senescing (Gonzalez-Aguilar *et al.*, 1997). These might be the reasons for increase in leaf chlorophyll content by the application of putrescine and IBA.

Shraiy and Hegazi (2009) conducted an experiment to study the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased total chlorophyll in leaves.

Ahmed *et al.* (2013) conducted a field experiment to study the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly enhanced chlorophyll content.

4.3.2 Leaf nitrogen content

Nitrogen is key component in mineral fertilizers and has more influence on plant growth, appearance and fruit production or quality than any other essential elements. Nitrogen is an important constituent of protein and protoplasm and essential for the growth of plants. Its storage leads to chlorosis and stoppage of growth and its presence in moderate doses is essential for plant growth and fruiting. An abundant supply of essential nitrogenous compound is required in each plant cell for normal cell division, growth and respiration. The N present mostly as protein is constantly moving and under concentration of N is found in young, tender plant tissues like tips of shoots, buds and new leaves (Jain, 2010).

It is observed from the data that there was significant variation in leaf nitrogen content due to foliar sprays of different concentrations of putrescine and IBA at 45, 60, 75 DAS except 30 DAS. Data are presented in table 10 and graphically illustrated in figure 11.

Table 10. Effect of putrescine and IBA on leaf nitrogen (%) content in soybean

Treatments	Leaf nitrogen content (%)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	1.50	2.38	2.30	1.89
T_2 (50 ppm Putrescine)	1.52	2.42	2.34	1.94
T_3 (75 ppm Putrescine)	1.48	2.57	2.43	2.01
T_4 (100 ppm Putrescine)	1.51	2.64	2.50	2.11
T_5 (125 ppm Putrescine)	1.54	2.48	2.38	1.97
T_6 (150 ppm Putrescine)	1.49	2.44	2.35	1.95
T_7 (50 ppm IBA)	1.48	2.42	2.31	1.90
T_8 (75 ppm IBA)	1.50	2.51	2.41	1.99
T_9 (100 ppm IBA)	1.55	2.61	2.49	2.08
T_{10} (125 ppm IBA)	1.53	2.44	2.35	1.94
T_{11} (150 ppm IBA)	1.52	2.43	2.32	1.91
SE (m) ±	0.079	0.052	0.033	0.041
CD at 5%	-	0.153	0.099	0.123

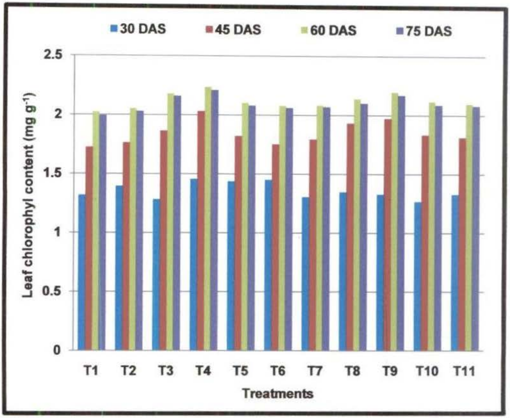


Figure 10. Effect of putrescine and IBA on leaf chlorophyll content (%) in soybean

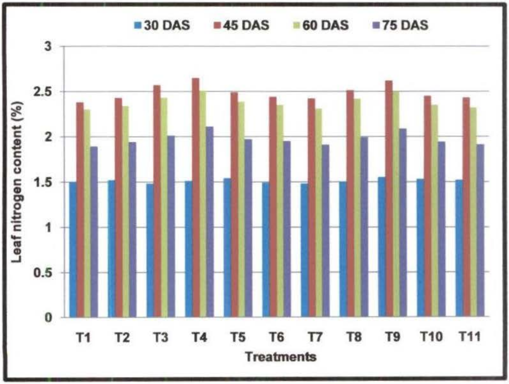


Figure 11. Effect of putrescine and IBA on leaf nitrogen content (%) in soybean

At 45 DAS nitrogen content was significantly more in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_7 (50 ppm IBA) and T_2 (50 ppm putrescine) were found at par with treatment T_1 (control). The range of N content at 45 DAS in soybean was 2 .38-2.64 %.

At 60 DAS nitrogen content was significantly maximum in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) in a descending manner when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_5 (125 ppm putrescine), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of N content at 60 DAS in soybean was 2 .30-2.50 %.

At 75 DAS nitrogen content was significantly maximum in treatment T_4 (100 ppm putrescine) followed by the treatment T_9 (100 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_3 (75 ppm putrescine), T_8 (75 ppm IBA) T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of N content at 75 DAS in soybean was 1.89-2.11%.

From this data it is observed that leaf nitrogen content was increased upto 60 DAS and reduced thereafter at 75 DAS. The decrease in nitrogen content might be due to fact that younger leaves and developing organs, such as seeds act as strong sink demand and may draw heavily nitrogen from older leaves (Gardner *et al.*, 1988).

Putrescine or IBA enhances enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amin *et al.*, 2013). Similarly IBA increases the ability of cell division in meristematic zones of plant and hence the ability of plant to absorb nutritive material (Ghodrat *et al.*, 2012). These might be the reasons for increase in leaf nitrogen content in the present investigation by the application of putrescine and IBA

Amin *et al.* (2013) tester two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly enhanced nitrogen of chickpea (*Cicer arientinum* L.)

Ahmed *et al.* (2013) conducted a field experiment to study the effect of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2 %) were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly enhanced inorganic nitrogen.

4.3.3 Leaf phosphorus content

Phosphorus is an important constituent of protoplasm and nucleic acid and protein also, it is essential for the formation of seed.

Data pertaining to phosphorus content in leaves were estimated at four stages of observations i.e. 30, 45, 60 and 75 DAS. Phosphorus has been recognized as an important environmental factor limiting crop growth and production. Significant results were recorded at all the stages of observations viz., 45, 60 and 75 DAS except 30 DAS. Data are presented in table 11 and figure 12.

At 45 DAS significantly more leaf phosphorus content was recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and other remaining treatments

Table 11. Effect of putrescine and IBA on leaf phosphorus content (%) in soybean

Treatments	Leaf phosphorus content (%)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	0.32	0.39	0.59	0.44
T_2 (50 ppm Putrescine)	0.35	0.42	0.65	0.51
T_3 (75 ppm Putrescine)	0.34	0.49	0.70	0.58
T_4 (100 ppm Putrescine)	0.37	0.54	0.77	0.60
T_5 (125 ppm Putrescine)	0.31	0.46	0.67	0.55
T_6 (150 ppm Putrescine)	0.33	0.43	0.66	0.52
T_7 (50 ppm IBA)	0.32	0.40	0.62	0.49
T_8 (75 ppm IBA)	0.36	0.45	0.68	0.54
T_9 (100 ppm IBA)	0.34	0.50	0.71	0.59
T_{10} (125 ppm IBA)	0.37	0.43	0.64	0.52
T_{11} (150 ppm IBA)	0.35	0.41	0.62	0.50
SE (m) ±	0.017	0.023	0.029	0.027
CD at 5%	-	0.069	0.086	0.082

under study. Treatment T_5 (125 ppm putrescine) also showed their significance over treatment T_1 (control). While, treatments T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of phosphorus content at 45 DAS in soybean was 0.39-0.54 %.

At 60 DAS significantly maximum leaf phosphorus content was recorded in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of phosphorus content at 60 DAS in soybean was 0.59-0.77%.

At 75 DAS leaf phosphorus content was significantly maximum in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_5 (125 ppm putrescine) and T_8 (75 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of phosphorus content at 75 DAS in soybean was 0.44-0.60%.

The interference drawn from the data, it is clear that leaf phosphorus content was gradually increase upto 60 DAS and reduced thereafter at 75 DAS. The increase in phosphorus content might be because of translocation of leaf phosphorus and it's utilization for development of food storage organ.

Application of putrescine or IBA increased enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amin *et al.*, 2013). These might be the reasons for increase in leaf phosphorus content in the present investigation by the application of putrescine and IBA

Amin *et al.* (2013) tester two plant growth regulators viz., putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1}, applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly enhanced phosphorus of chickpea (*Cicer arientinum* L.).

4.3.4 Leaf potassium content

Potassium is an essential macronutrient for plants involved in many physiological processes. It is important for crop yield as well as for the quality of edible parts of crops. Although K is not assimilated into organic matter, K deficiency has a strong impact on plant metabolism. Plant responses to low K involve changes in the concentrations of many metabolites as well as alteration in the transcriptional levels of many genes and in the activity of many enzymes.

Data pertaining to potassium content in leaves were estimated at various stages of observations viz., 30, 45, 60 and 75 DAS. Significant results were recorded at all the stages of observations viz., 45, 60 and 75 DAS except 30 DAS. Data are presented in table 12 and figure 13.

At 45 DAS significantly maximum leaf potassium content was observed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA) and T_5 (125 ppm putrescine) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of potassium content at 45 DAS in soybean was 1.18-1.51%.

Table 12. Effect of putrescine and IBA on leaf potassium content (%) in soybean

Treatments	Leaf potassium content (%)			
	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (Control)	0.95	1.18	2.10	1.31
T_2 (50 ppm Putrescine)	0.94	1.27	2.19	1.39
T_3 (75 ppm Putrescine)	1.22	1.44	2.27	1.78
T_4 (100 ppm Putrescine)	1.23	1.51	2.40	1.87
T_5 (125 ppm Putrescine)	0.99	1.40	2.21	1.69
T_6 (150 ppm Putrescine)	1.08	1.31	2.20	1.48
T_7 (50 ppm IBA)	1.03	1.24	2.17	1.37
T_8 (75 ppm IBA)	0.97	1.41	2.24	1.71
T_9 (100 ppm IBA)	0.98	1.47	2.35	1.83
T_{10} (125 ppm IBA)	1.12	1.37	2.19	1.62
T_{11} (150 ppm IBA)	1.09	1.30	2.18	1.46
SE (m) ±	0.074	0.066	0.052	0.083
CD at 5%	-	0.197	0.154	0.247

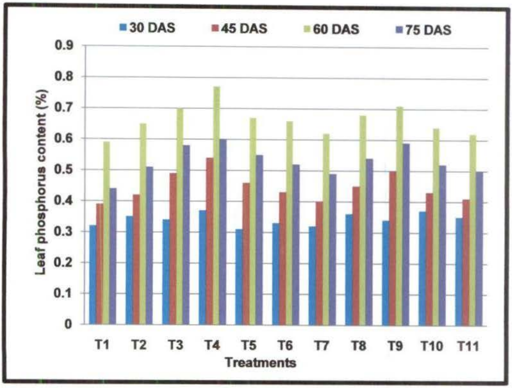


Figure 12. Effect of putrescine and IBA on leaf phosphorus content (%) in soybean

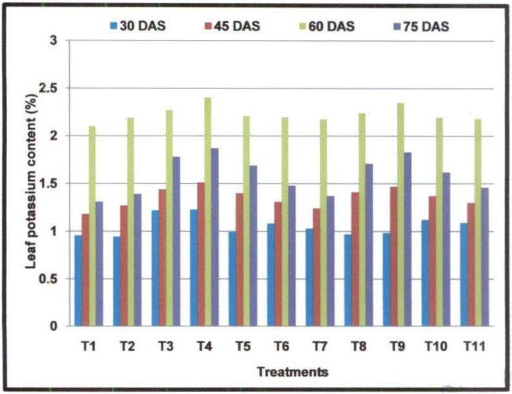


Figure 13. Effect of putrescine and IBA on leaf potassium content (%) in soybean

At 60 DAS significantly maximum leaf potassium content was observed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of potassium content at 60 DAS in soybean was 2.10-2.40%.

At 75 DAS significantly maximum leaf potassium content was observed in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA), T_5 (125 ppm putrescine) and T_{10} (125 ppm IBA) in a descending manner when compared with treatment T_1 (control) and remaining treatments under study. While, treatments T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_2 (50 ppm putrescine) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control). The range of potassium content at 75 DAS in soybean was 1.31-1.87%.

It is from the data, that leaf potassium content was increased gradually upto 60 DAS and decreased at 75 DAS. It might be because of diversion of potassium towards developing parts i.e. pods of the soybean crop at advanced stage.

Application of putrescine or IBA increased enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amin *et al.*, 2013). These might be the reasons for increase in leaf potassium content in the present investigation by the application of putrescine and IBA

El-Bassiouny *et al.* (2008) tried arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and found that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased potassium.

Amin *et al.* (2013) tester two plant growth regulators i.e. putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1}, applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly enhanced potassium of chickpea (*Cicer arientinum* L.).

4.3.5 Protein content in seed

Protein content of the seed is one of the considerable factor for seed quality determination. The table 13 gives detail data on protein content and it is presented graphically by figure 14.

Data indicated that protein content was significantly increased in treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatment under study. While,treatments T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

Foliar application of putrescine and IBA increased seed protein content in the present investigation might be due to enhances enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amin, 2013).

Shraiy and Hegazi (2009) conducted an experiment to study the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased protein content.

Mathur and Vyas (2007) conducted an experiment to study the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum*

Table 13. Effect of putrescine and IBA on seed protein and oil content (%) in soybean

Treatments	Protein content (%)	Oil content (%)
T_1 (Control)	35.91	19.81
T_2 (50 ppm Putrescine)	37.66	20.99
T_3 (75 ppm Putrescine)	39.57	21.88
T_4 (100 ppm Putrescine)	41.97	22.75
T_5 (125 ppm Putrescine)	38.02	21.22
T_6 (150 ppm Putrescine)	37.78	21.00
T_7 (50 ppm IBA)	37.32	20.17
T_8 (75 ppm IBA)	38.83	21.39
T_9 (100 ppm IBA)	40.34	22.24
T_{10} (125 ppm IBA)	37.97	20.98
T_{11} (150 ppm IBA)	37.42	20.29
SE (m) ±	1.083	0.533
CD at 5%	3.200	1.575

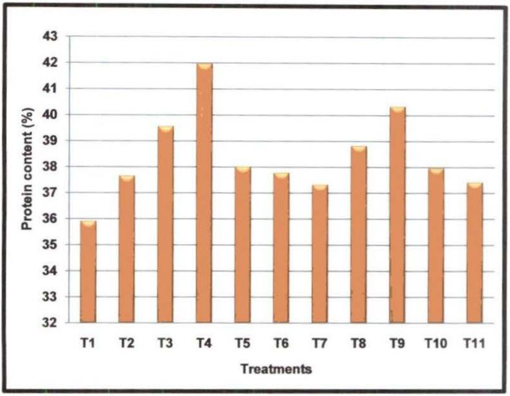


Figure 14. Effect of putrescine and IBA on seed protein content (%) in soybean

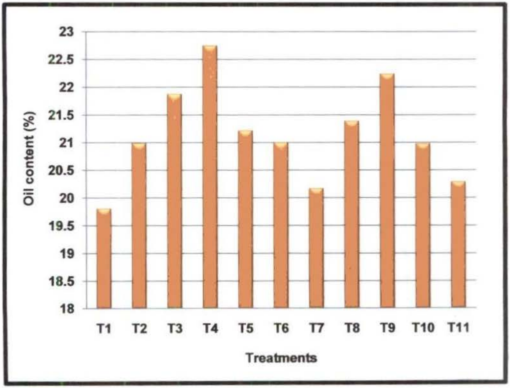


Figure 15. Effect of putrescine and IBA on seed oil content (%) in soybean

typhoides). Result showed that application of salicylic acid at 3 mM and sitosterol or putrescine at 0.15 mM significantly increased crude protein.

4.3.6 Oil content in seed

Soybean is mainly known as oilseed crop and oil percentage in seed is one of the important aspect in quality of grain. The data regarding oil content of seed are given table 13 and graphically presented by figure 15.

Maximum oil content in seed of soybean was recorded in the treatment T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. The treatments T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_2 (50 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with treatment T_1 (control).

Data revealed that, foliar application of 100 ppm putrescine stood first in oil content in soybean. Oil content in soybean seed recorded in treatment T_1 (control) was 19.81 % and in treatment T_4 (100 ppm putrescine) was 22.75 %.

It appeared from the results that putrescine or IBA are more effective on seed oil content. This might be due to enhancement of enzymatic activity and translocation of metabolites to the soybean seed.

Mathur and Vyas (2007) conducted an experiment to study the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Results showed that application of salicylic acid at 3 mM and sitosterol or putrescine at 0.15 mM significantly increased oil content.

4.4 Yield and yield contributing parameters

Seed yield and its related parameters in soybean were influenced by the application of growth regulator which have different influence on the allocation of assimilates between vegetative and reproductive organs. In general crop yield depends on the accumulation of photo-assimilates during the growing period and the way, they are partitioned between desired storage organ of plant. In present study, it was revealed that the application of plant growth regulator significantly increased the number of seeds, number of pods, 100 seed weight and finally seed yield determining components in soybean.

4.4.1 Number of pods plant^{-1}

The data with respect to number of pods plant^{-1} are presented in table 14 and depicted graphically in figure 16.

Data indicates that all the treatments were statistically significant over control. Number of pods plant^{-1} were significantly increased in treatment T_4 (100 ppm putrescine) followed by treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_5 (125 ppm putrescine) in a descending manner when compared with remaining treatments and treatment T_1 (control). Treatments T_8 (75 ppm IBA) and T_{10} (125 ppm IBA) also showed their significance over treatment T_1 (control). While, treatments T_6 (150 ppm putrescine), T_{11} (150 ppm IBA), T_2 (50 ppm putrescine), and T_7 (50 ppm IBA) were found at par with T_1 (control).

Thavaprakash *et al.* (2006) conducted an experiment to study the effect of plant growth promoters on assimilate partitioning and seed yield of green gram. The results revealed that foliar application of putrescine and spermine @ 20 ppm registered significant increase in yield attributing characters such as numbers of pods plant^{-1} over control.

Table 14. Effect of putrescine and IBA on number of pods plant^{-1} and 100 seed weight in soybean

Treatments	No. of pods plant^{-1}	100 seed weight (g)
T_1 (Control)	82.13	8.61
T_2 (50 ppm Putrescine)	97.46	9.52
T_3 (75 ppm Putrescine)	123.20	10.79
T_4 (100 ppm Putrescine)	132.86	11.69
T_5 (125 ppm Putrescine)	115.46	10.72
T_6 (150 ppm Putrescine)	100.53	9.82
T_7 (50 ppm IBA)	93.53	10.32
T_8 (75 ppm IBA)	110.46	10.47
T_9 (100 ppm IBA)	128.46	11.11
T_{10} (125 ppm IBA)	109.06	10.36
T_{11} (150 ppm IBA)	100.46	10.06
SE (m) ±	6.591	0.305
CD at 5%	19.47	0.902

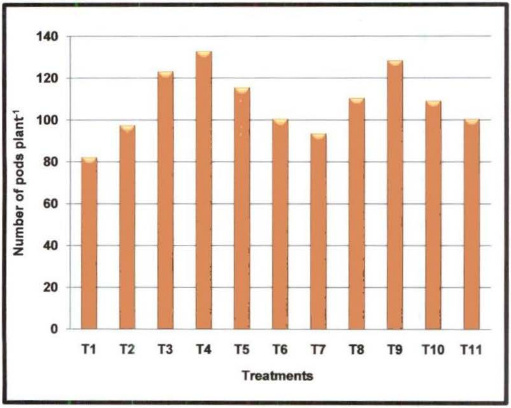


Figure 16. Effect of putrescine and IBA on number of pods plant^{-1} in soybean

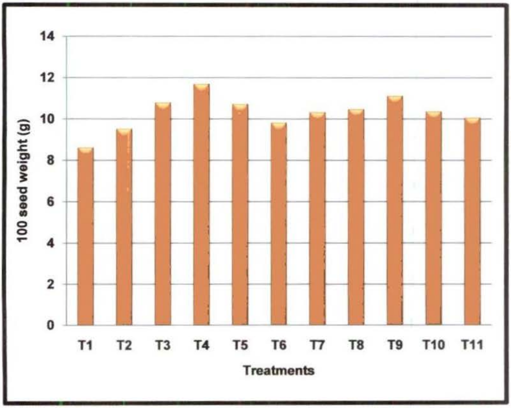


Figure 17. Effect of putrescine and IBA on 100 seed weight in soybean

Shraiy and Hegazi (2009) studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased number of pods plant^{-1}.

Amin *et al.* (2013) studied the effect of two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased number of pods of chickpea (*Cicer arientinum*).

4.4.2 Weight of 100 seeds

Data regarding 100 seed weight showed significant variation and are given in table 14 and figure 17. It was observed that foliar application of 100 ppm putrescine gave significantly higher 100 seed weight when compared with treatment T_1 (control) and remaining treatments under study. The range of increase in seed weight was 8.61 g in control (T_1) to 11.69 g in treatment receiving 100 ppm putrescine (T_4).

100 seed weight was also significantly increased in treatment receiving 100 ppm putrescine followed by treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. Treatments T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_7 (50 ppm IBA), T_{11} (150 ppm IBA), T_6 (150 ppm putrescine) and T_2 (50 ppm putrescine) also showed their significance over treatment T_1 (control).

El-Bassiouny *et al.* (2008) tested arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and reported that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased 1000 grains weight over control.

Shraiy and Hegazi (2009) studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased 1000 seeds weight over control.

4.4.3 Seed yield plant^{-1} (g), plot^{-1} (kg) and ha^{-1}(q)

Data regarding seed yield plant^{-1}, plot^{-1}, ha^{-1} are given in table 15 and figure 18, 19 and 20 respectively.

Seed yield is a complex physiological character which is the sum total of all metabolic activities taking place in plant body. This includes various morphological aspects like increase in plant height, leaf size, leaf area, number of branches, dry matter, seed weight etc. These characters can be considered as yield contributing parameters.

Seed yield and its related parameters were influenced by the application of different growth regulators in soybean which indicated that these chemicals have differential influence on the allocation of assimilates between vegetative and reproductive organs. In general, crop yield depends on the accumulation of photo-assimilates during the growing period and the way they are partitioned between desired storage organs of plant. In the present study, it is revealed that the application of PGRs significantly increased the number of pods, 100-seed weight and finally seed yield plant^{-1}, plot^{-1} and hectare^{-1} which are the most important yield determining components in soybean.

The maximum grain yield plant^{-1}, plot^{-1}, hectare^{-1} were recorded in treatment T_4 (100 ppm putrescine). The range of increase in seed yield plant^{-1} , plot^{-1} and hectare^{-1} was 5.26 g, 0.52 kg and 17.84 q in treatment T_1 (control) to 6.70 g, 0.67 kg and 22.46 q in treatment T_4 (100 ppm putrescine) respectively.

Significantly maximum seed yield plant^{-1}, plot^{-1}, ha^{-1} were recorded in treatment T_4 (100 ppm putrescine) when compared with control and remaining treatments under study. Next to this treatment,

Table 15. Effect of putrescine and IBA on seed yield plant^{-1} (g), plot^{-1} (kg), ha^{-1} (q), Per cent increase in yield and harvest index in soybean

Treatments	Seed yield plant^{-1} (g)	Seed yield plot^{-1} (kg)	Seed yield ha^{-1} (q)	Per cent increase in yield	Harvest Index (%)
T_1	5.26	0.52	17.84	-	31.07
T_2	5.54	0.55	18.58	04.14	36.97
T_3	5.99	0.59	20.07	12.50	40.11
T_4	6.70	0.67	22.46	25.89	45.22
T_5	5.94	0.59	19.90	13.45	39.52
T_6	5.80	0.57	19.44	08.96	35.63
T_7	5.47	0.54	18.35	02.85	32.50
T_8	5.84	0.58	19.58	09.75	39.62
T_9	6.41	0.64	21.47	20.34	40.82
T_{10}	5.76	0.57	19.29	08.12	38.02
T_{11}	5.69	0.56	19.07	06.89	35.47
SE(m)±	0.071	0.007	0.266	-	1.99
CD at 5%	0.210	0.023	0.787	-	5.88

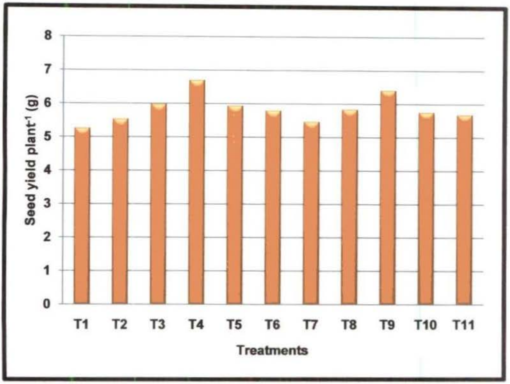


Figure 18. Effect of putrescine and IBA on seed yield plant^{-1} (g) in soybean

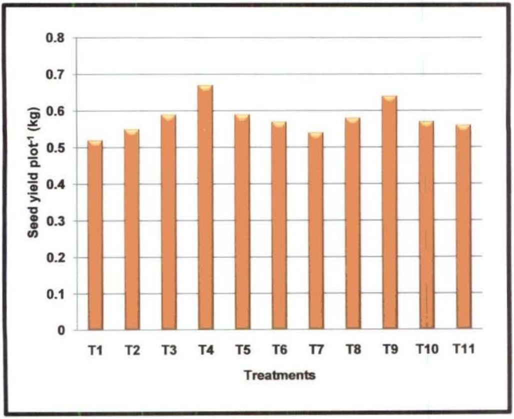


Figure 19. Effect of putrescine and IBA on seed yield plot^{-1} (kg) in soybean

treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA) and T_2 (50 ppm putrescine) also gave maximum seed yield plant^{-1}, plot^{-1}, ha^{-1} when compared with treatment T_1 (control). While, treatment T_7 (50 ppm IBA) was found at par with T_1 (control).

Plant growth regulators are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and enhance the effective partitioning of accumulates from source and sink in the field crops (Solamani *et al.*, 2001).

The diamine putrescine occurred widely in the higher plants.It was suggested to be involved in a variety of growth and developmental processes such as cell division (Bueno and Matilla,1992), fruit set and growth (Biasi *et al.*,1991) and senescence (Kao,1994).The interaction of polyamines with the macromolecules was responsible for physiological effects on plant growth and development (Smith,1985).

Growth regulator IBA is proved to improve effective partitioning and translocation of accumulates from source to sink in the field crops. The plant growth regulators also increase mobilization of reserve food materials to the developing sink through increases in hydrolyzing and oxidizing enzyme activities and lead to yield increases. IBA increases the ability of cell division in meristematic zones of plant and hence the ability of plant to absorb nutritive material which finally lead to the increase of grain yield (Ghodrat *et al.*, 2012).

The increase in the yield recorded in this investigation could be a reflection of the effect of growth regulators on growth and development, it might be due to marked increase in plant height, leaf area, dry weight, number of branches which gave a chance to the plant

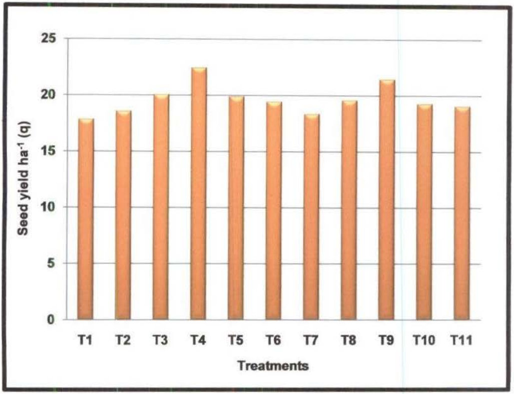


Figure 20. Effect of putrescine and IBA on seed yield ha^{-1} (q) in soybean

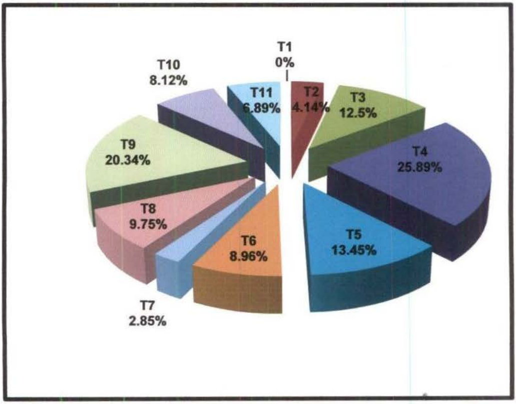


Figure 21. Effect of putrescine and IBA on per cent increase in yield in soybean

to carry more seed and marked increase in the photosynthetic pigments content which could lead to increase in photosynthesis, resulting in greater transfer of assimilates to the seeds and causing increase in their weight.

Thavaprakash *et al.* (2006) conducted an experiment to study the effect of plant growth promoters on assimilate partitioning and seed yield of green gram. The results revealed that foliar application of putrescine and spermine @ 20 ppm registered significant increase in yield attributing characters such as numbers of flowers plant^{-1}, number of pods plant^{-1}, fertility coefficient, number of filled seeds pod^{-1} and per cent filled seeds over control.

Field experipent was conducted by Mathur and Vyas (2007) to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on pearl millet (*Pennisetum typhoides*). Results showed that application of salicylic acid @ 3 mM and sitosterol or putrescine @ 0.15 mM significantly increased yield and its components i.e. ear length, ear diameter, grain yield plant^{-1}, crop index and 100- grain weight over control.

Amin *et al.* (2013) studied the effect of two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased number of pods, seed yield, straw and biological yield feed^{-1} of chickpea (*Cicer arientinum*).

4.4.4 Harvest index

Data regarding harvest index are given in table 15 and graphically represented in figure 22.

Data were found statistically significant. Significantly maximum harvest index was recorded in treatment T_4 (100 ppm putrescine) and minimum in control. The range of increased harvest index was 31.07 in control to 45.22 % in above treatments.

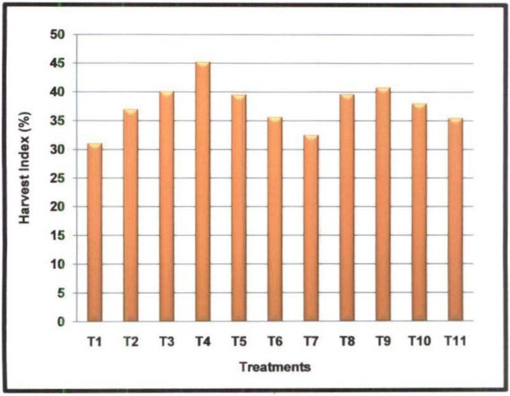


Figure 22. Effect of putrescine and IBA on Harvest Index (%) in soybean

Similarly, harvest index was also significantly increased in treatments receiving T_4 (100 ppm putrescine) followed by the treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA) and T_5 (125 ppm putrescine) when compared with control and remaining treatments under study. Treatments T_{10} (125 ppm IBA) and T_2 (50 ppm putrescine) were also found significantly superior over treatment T_1 (control). While, treatments T_6 (150 ppm putrescine), T_{11} (150 ppm IBA) and T_7 (50 ppm IBA) were found at par with T_1 (control).

El-Bassiouny *et al.* (2008) tested arginine and putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) and reported that foliar application of 2.5 mM arginine and putrescine on wheat significantly increased straw yield and harvest index when applied at 30 or 60 DAS over control.

Harvest index is the proportion of biological yield represented by economic yield. It is the coefficient of effectiveness or migration coefficient. Harvest index reflects the proportion of assimilate distribution between the economic and total biomass (Donald and Hamblin, 1976). Increase in harvest index might be the result of co-ordinated interplay of growth and development characters.

The highest per cent increase in yield over control was observed in treatment sprayed with 100 ppm putrescine i.e. 25.89 %. Next to this treatment foliar spray of 100 ppm IBA also enhanced yield by 20.34 % over control.

From overall results, it can be stated that foliar application of growth regulators such as putrescine and IBA with different concentrations improved the morpho-physiological, biochemical and yield and yield contributing parameters might have helped in attaining better seed yield in the present investigation.

Chapter V

SUMMARY AND CONCLUSIONS

The present investigation was undertaken during the *kharif* season of 2014-2015 to study the "Influence of putrescine and indole-3-butyric acid on growth and productivity of soybean" JS-335. The experiment was laid out in randomized block design with eleven treatments and three replications at farm of Botany section, College of Agriculture, Nagpur.

Plot size of individual treatment was gross 2.10 m x 2.2 m and net 1.5 m x 2.0 m. Seeds were sown at the rate of 75 kg ha^{-1} by dibbling method at spacing of 30 cm X 10 cm on 14^{th} July 2014. Treatments comprised of T_1 (control), T_2 (50 ppm putrescine), T_3 (75 ppm putrescine), T_4 (100 ppm putrescine), T_5 (125 ppm putrescine), T_6 (150 ppm putrescine), T_7 (50 ppm IBA), T_8 (75 ppm IBA), T_9 (100 ppm IBA), T_{10} (125 ppm IBA) and T_{11} (150 ppm IBA). The foliar application of putrescine and IBA was given at two stages i.e. before flowering (30DAS) and 10 days after flowering (45DAS) on soybean.

The object of the investigation was to study the effect different concentrations of putrescine and indole-3-butyric acid on morpho-physiological, chemical and biochemical, yield and yield contributing parameters of soybean.

The morpho-physiological observations viz., plant height, number of branches $plant^{-1}$, leaf area and dry matter production were recorded at 30, 45, 60 and 75 DAS. Similarly RGR and NAR were calculated at 30-45, 45-60 and 60-75 DAS. Leaf chlorophyll, nitrogen, phosphorus and potassium were analyzed at 30, 45, 60 and 75 DAS. Whereas, protein and oil content in seed were also estimated.

Observations on yield and yield contributing parameters like, number of pods $plant^{-1}$, 100 seed weight, seed yield $plant^{-1}$, seed yield $plot^{-1}$, and seed yield $hectare^{-1}$ were also recorded. Harvest Index was also worked out. The data collected were subjected to statistical analysis and the inferences drawn are summarized as under.

Plant height was recorded at 30, 45, 60 and 75 DAS. Foliar application of 100 ppm putrescine (T_4) and 100 ppm IBA (T_9) were found superior among all other treatments and over control (T_1). The treatment T_8 (75 ppm IBA) was also increased plant height significantly over the other treatments and control (T_1).

Growth parameters like number of branches plant^{-1}, leaf area and dry matter production were noted at 30, 45, 60 and 75 DAS. Data revealed that plants sprayed with 100 ppm putrescine (T_4) and 100 ppm IBA (T_9) were found superior among all other treatments and control. The treatments T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) were also exhibited their significance over all other treatments and control (T_1).

Data regarding RGR and NAR at 30-45, 45-60 and 60-75 DAS revealed that foliar application of 100 ppm putrescine (T_4) was most effective than control and all other treatments. The treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_8 (75 ppm IBA), T_5 (125 ppm putrescine), T_{10} (125 ppm IBA), T_6 (150 ppm putrescine), T_7 (50 ppm IBA), T_2 (50 ppm putrescine) and T_{11} (150 ppm IBA) also showed their significance over other treatments and control (T_1).

The biochemical parameter such as leaf chlorophyll was noted at 30, 45, 60 and 75 DAS. Data revealed that plants sprayed with 100 ppm putrescine (T_4) and 100 ppm IBA (T_9) were found superior among all other treatments and over control.

The chemical parameters like as nitrogen, phosphorus and potassium were significantly more with the foliar application of 100 ppm putrescine (T_4). The treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) also showed their significance over other treatments and control (T_1) in a descending order.

The maximum seeds protein was recorded with the foliar application of 100 ppm putrescine (T_4) which was found superior among all other treatments and over control. The treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) also increased the seed protein content over the other treatments and control (T_1).

The maximum oil content was recorded with the foliar application of 100 ppm putrescine (T_4) which was found superior among all other treatments and over control. The treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine) and T_8 (75 ppm IBA) were also increased oil content over the other treatments and control (T_1).

Yield contributing characters such as number of pods plant^{-1} significantly increased by the foliar application of 100 ppm putrescine (T_4). The treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_5 (125 ppm putrescine), T_8 (75 ppm IBA) and T_{10} (125 ppm IBA) were also showed their significance over the other treatments and control (T_1).

100 seed weight was also significantly increased in treatment receiving 100 ppm putrescine followed by treatments T_9 (100 ppm IBA) and T_3 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. Treatments T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_{10} (125 ppm IBA), T_7 (50 ppm IBA), T_{11} (150 ppm IBA), T_6 (150 ppm putrescine) and T_2 (50 ppm putrescine) also showed their significance over treatment T_1 (control).

Significantly maximum seed yield plant^{-1}, plot^{-1}, ha^{-1} were recorded in treatment T_4 (100 ppm putrescine) when compared with control and remaining treatments under study. Next to this treatment, treatments T_9 (100 ppm IBA), T_3 (75 ppm putrescine), T_5 (125 ppm putrescine), T_8 (75 ppm IBA), T_6 (150 ppm putrescine), T_{10} (125 ppm IBA), T_{11} (150 ppm IBA) and T_2 (50 ppm putrescine) also gave maximum seed yield plant^{-1}, plot^{-1}, ha^{-1} when compared with treatment T_1 (control).

- From the above data following inferences can be drawn.

- Soybean responded to the foliar application of putrescine and indole-3- butyric acid and gave significant variation among different characters studied.

- Considering the different concentrations applied, foliar sprays of 100 ppm putrescine and 100 ppm IBA at two stages i.e. before flowering (30DAS) and 10 days after flowering (45DAS) stood first and second in rank and significantly enhanced all parameters under study when compared with control and rest the treatments under study.

- From the present study, it can be inferred that foliar application of 100 ppm putrescine followed by 100 ppm IBA at two stages i.e. before flowering (30DAS) and 10 days after flowering (45DAS) significantly enhanced the morpho-physiological, chemical and biochemical, yield and yield contributing parameters and ultimately increased the yield by 25.89 followed by 20.34 per cent respectively over control in soybean.

- Above inferences are drawn on the basis of one year data.

Chapter VI

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1.	B.Sc. (Agril.)	2013	First	Dr. P.D.K.V. Akola	Agriculture
2.	HSC	2009	First	Amravati	Science
3.	SSC	2007	First	Amravati	General

6. Research paper published if any : Process

7. Field of interest: Research work in Agriculture Sector.

Place: Nagpur

Date: *14/05 /2015*

Signature of student
(Wagh Yogesh Ashokrao)

APPENDIX

A. Estimation of total chlorophyll content

- Accurately weighed 500 mg of over dried sample was taken and transferred to morter and 2 ml of 80% acetone was added and homogenized to form a paste.

- The chlorophyll extract was obtained.

- The extraction was carried out by giving subsequent washing of 10 ml of 80 % acetone every time till leachate was colourless. The final volume was made to 50 ml in each volumetric flask by using 80 % acetone. Whatman filter paper number 40 was used for filtration.

- The optical density of the extract was measured on double photocell colorimeter by using red filter. Total chlorophyll content (mg g^{-1}) of the dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982).

B. Estimation of nitrogen content in leaves

- Reagents used

- Salt mixture

- 20 g $CuSO_4 . H_2O$ + 20 g $Na_2 SO_3$ + 0.5 g selenium powder

- 0.1 N HCL: It was prepared by diluting 8.5 ml concentrated hydrochloric acid in little quantity of distilled water and volume is made up to 1 litre.

- 0.1 Boric acid: It was prepared by dissolving 20 g of boric acid powder in little quantity of distilled water and volume was made up to 1 litre.

- Methyl orange indicator: 0.5 g of powder was dissolved in 500 ml alcohol and 500 ml distilled water.

- Methyl red indicator: 2 g powder of methyl red was dissolved in 400 ml alcohol.

- The nitrogen content in leaves was determined by Micro-kjeldhal's method given by Somichi *et al.* (1972). 200 mg oven dried finely ground plant sample was weighted accurately on electronic balance and transferred to digestion flask to which 5 ml of concentrated H_2SO_4 and a pinch of salt mixture of $CuSO_4$ (20 g) selenium powder (0.5 g) and $Na_2\ SO_3$ (20 g) were added and then flask were kept in the digestion chamber till clear transparent liquid was obtained. The flasks were then cooled and 10 ml of distilled water was added in it.

- The distillation was carried out by adding 10 ml of saturated N_aOH solution in distillation apparatus. The NH_3 was collected in 0.1 N 25 ml of boric acid solution to which mixed indicator (methyl red + methyl orange) was added.

- The distillation was continued till red litmus paper did not change the colour to blue.

- The boric acid solution was then titrated against 0.1 N HCL. The percentage of nitrogen in sample was calculated.

C. Estimation of P content in leaves

- Reagents used

- 9:4 acid mixture (90 ml HNO_3 + 40 ml perchloric acid)

- 25 g - Ammonium molybdate

- 1.25 g - Ammonium metavanadate

- 0.2195 g Analytical grade KH_2PO_4

- The phosphorus and potassium content in leaves was determined by vanadomolybdate yellow colour method given by Jackson (1967). 1 g oven dried plant sample was weighed accurately on electronic balance and then transfer to 100 ml conical flask to which 10 ml acid mixture (9: 4) was added.

- Conical flask were kept on sand bath and heated at higher temperature until the red fumes ceases or acid mixture turn in to white colour.

- The flasks were then cooled for half an hour and then 20 ml distilled water was added and then filter through filter paper.

- Volume was made up to 100 ml in volumetric flask (aliquate) and out of this 100 ml aliquate 10 ml were taken in 50 ml round bottom flask. In round bottom flask 10 ml vanodomolybdate mixture was added and volume was made up to 50 ml with distilled water.

- From this solution, working solutions were prepared of concentrations of 0, 1, 2, 3, 4, 5 ppm standard solution.

- Colour intensity was recorded by using colorimeter from working solutions and plant samples.

- Standard curve was drawn with colorimeter reading on y axis and ppm value on x axis.

D. Estimation of K content in leaves

Reagents used

1) 9:4 diacid mixture (HNO_3 : Perchloric acid).

2) KCl 1.908 g in 1 lit. of distilled water.

- Plant sample digested with diacid was used for potassium estimation also. Aliquot taken from the filtered clear solution was used for K estimation and read in flame photometer.

- Along with this, standard K solution was prepared by dissolving 1.908 g of analytical grade KCl in 1 lit. of water. From this, working solutions of 0, 10, 20, 40, 60 and 80 ppm K were prepared to calibrate the instrument. Readings were noted and a curve was drawn. A blank was also run.

- From the graph, reading was noted down for the sample. (Jackson, 1967).

E. Estimation of protein content in seed

Nitrogen content in seeds was determined by micro-kjeldahl's method (Somichi *et al.*, 1972) and the same was converted to crude protein by multiplying N percentage with the factor 5.76.

F.. Estimation of oil content in seeds

- 2 g of oven dried, crushed seeds were weighted accurately on electronic balance and wrapped in double wrapper of filter paper in such a manner to give conical shape.

- These were then placed in thimble and were put in Soxhlet's extraction apparatus connected pre-weighed 150 ml extraction flask.

- They were filled with 11 cycles petroleum ether solvent (boiling point 40-60^{0}c). The condensers in the assembly were connected to a continuous stream of cold water.

- These extraction flasks were heated until eight complete cycles were run to give adequate time to remove the last traces of crude fat.

- After the last cycle was completed, the petroleum ether was collected in thimble and transfer to beaker.
- The flasks were detached from assembly and transfer to an oven at 100–105^{0}c for complete removal of petroleum ether. The flask were weighted and calculate per cent oil content.