IDENTIFICATION OF MAIZE GENOTYPES TOLERANT TO WATERLOGGING AND THEIR CHARACTERIZATION USING RICE ORTHOLOGS

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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in
PLANT BREEDING AND GENETICS
(Minor Subject: Biotechnology)

By

Sittal Thapa
(L-2019-A-105-M)

Department of Plant Breeding and Genetics
College of Agriculture

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CERTIFICATE I

This is to certify that the thesis entitled, **"Identification of maize genotypes tolerant to waterlogging and their characterization using rice orthologs"** submitted for the degree of **M.Sc.,** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Sittal Thapa (L-2019-A-105-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

(Dr Tosh Garg)
Major Advisor
Maize Breeder
Department of Plant Breeding and Genetics, PAU, Ludhiana

CERTIFICATE-II

This is to certify that the thesis entitled, **"Identification of maize genotypes tolerant to waterlogging and their characterization using rice orthologs"** submitted by **Sittal Thapa (L-2019-A-105-M)** to the Punjab Agricultural University, Ludhiana in partial fulfillment of the requirements for the degree of **M.Sc.,** in the subject of **Plant Breeding and Genetics** (Minor Subject: **Biotechnology**) has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with External Examiner.

_____
(Dr Tosh Garg)
Major Advisor

_____
(Dr Pardeep Kumar)
External Examiner
Scientist (Plant Breeding)
ICAR- Indian Institute of Maize Research (IIMR), PAU Campus
Ludhiana - 141 004 (India)

_____
(Dr V.S. Sohu)
Head of the Department

_____
(Dr Jaskarn Singh Mahal)
Dean, Postgraduate Studies

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ABSTRACT

Waterlogging, second most important abiotic constraint after drought, leads to around 25-30% of production losses each year. Identification of suitable donor lines with tolerance against waterlogging and their genetic and morphological characterization is essential to develop varieties that can overcome the ill effects posed by excess soil moisture. The present study focused on identification of maize genotypes tolerant to waterlogging and their characterization using rice orthologs. A set of 119 different genotypes belonging to different heterotic pools along with *Zea mays spp parviglumis* were screened at pre-emergence and seedling stage (V_2) against different duration of waterlogging. In response to waterlogging all morphological parameters showed significant decline over increased duration of waterlogging. Based on selection indices, multi trait stability index (MTSI) and survival data after nine days of waterlogging, lines selected for tolerance against waterlogging are EML 153, SE 503, EML 125, AVL13119, VL 1010764, I 168, I 204, AVL13056, PML 368, LM 17, *Z. mays spp parviglumis*, PML 373, PML 387, EML 285, JCY 20, I 170, I 185, I 172, PML 1228, PML 243, PML 28, EML 124, LM 6, I 185 and I 192.Waterlogging tolerance coefficient (WTC) for tolerant lines lies near or above one. Broadened anthesis silking interval (>5 days) was recorded for susceptible lines, while for tolerant lines it lie within the normal range of 3-5 days. Search for ortholog genes responsible for submergence tolerance in rice revealed no significant region for *SNORKEL*, but significant sequence similarity for two out of three coding sequences for *Sub1A*. Synthesis of gene specific primers and allelic survey study showed no significant allelic variation for these genes in studied genotypes. Gene expression study revealed significantly higher fold expression of these genes in tolerant lines (up to 15 folds) during early stage of waterlogging (3 days of waterlogging) suggesting their involvement in conferring tolerance against anaerobic growth condition. Population structure analysis revealed presence of three sub-populations in our available germplasm. An average Polymorphism Information Content (PIC) value of 0.503 was obtained for SSR markers used for the genetic diversity study. Marker Trait Association (MTA) revealed two markers umc-1021 and umc-2340 to be significantly associated with both root area ($-\log_{10}p$ of 3.91) and root volume ($-\log_{10}p$ of 11.34).

Keywords: Waterlogging, MTSI, WTC, Rice orthologs, Expression analysis, MTA

Signature of Major Advisor **Signature of student**

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ਸੋਕੇ ਤੋਂ ਬਾਅਦ ਪਾਣੀ ਦਾ ਭਰਾਅ ਇੱਕ ਅਜਿਹੀ ਅਜੈਵਿਕ ਸਮੱਸਿਆ ਹੈ ਜਿਸ ਕਾਰਨ ਹਰ ਸਾਲ ਫ਼ਸਲ ਦੇ ਉਤਪਾਦਨ ਦਾ 25-30% ਨੁਕਸਾਨ ਹੁੰਦਾ ਹੈ। ਪਾਣੀ ਦੇ ਭਰਾਅ ਦਾ ਟਾਕਰਾ ਕਰਨ ਦੇ ਸਮਰੱਥ ਅਨੁਕੂਲ ਡੋਨਰ ਲਾਈਨਾਂ ਦੀ ਪਹਿਚਾਣ ਅਤੇ ਉਹਨਾਂ ਦਾ ਅਨੁਵਾਂਸ਼ਿਕੀ ਅਤੇ ਆਕ੍ਰਿਤਕ ਚਿੱਤਰ-ਚਿਤਰਣ ਬਹੁਤ ਜਰੂਰੀ ਹੈ ਤਾਂ ਜੋ ਮਿੱਟੀ ਵਿੱਚ ਵਾਧੂ ਨਮੀ ਕਾਰਨ ਫ਼ਸਲ ਨੂੰ ਹੋਣ ਵਾਲੇ ਨੁਕਸਾਨ ਉਪਰ ਕਾਬੂ ਪਾਇਆ ਜਾ ਸਕੇ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ ਪਾਣੀ ਦੇ ਭਰਾਅ ਦਾ ਟਾਕਰਾ ਕਰਨ ਵਾਲੇ ਮੱਕੀ ਦੇ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਪਹਿਚਾਣ ਕੀਤੀ ਗਈ ਅਤੇ ਝੋਨੇ ਦੇ ਔਰਥੋਲਗਸ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਉਹਨਾਂ ਦਾ ਚਿੱਤਰ-ਚਿਤਰਣ ਕੀਤਾ ਗਿਆ। ਵੱਖੋ-ਵੱਖਰੇ ਪਾਣੀ ਦੇ ਭਰਾਅ ਅੰਤਰਾਲਾਂ ਵਿਰੁੱਧ, ਅੰਕੁਰਤ ਹੋਣ ਤੋਂ ਪਹਿਲਾਂ ਅਤੇ ਜ਼ਮੀਨ ਵਿੱਚੋਂ ਪੌਦੇ ਦੇ ਨਿਕਲਣ ਦੀ ਅਵਸਥਾ (V_2) ਉਪਰ ਵੱਖੋ-ਵੱਖਰੇ ਹੈਟ੍ਰੋਟਿਕ ਪੂਲਾਂ ਨਾਲ ਸਬੰਧਤ ਕੁੱਲ 119 ਜੀਨੋਟਾਈਪਾਂ ਅਤੇ *Zea mays spp parviglumis* ਦੀ ਜਾਂਚ ਕੀਤੀ ਗਈ। ਪਾਣੀ ਦੇ ਭਰਾਅ ਦੇ ਅੰਤਰਾਲ ਵਿੱਚ ਵਾਧਾ ਹੋਣ ਨਾਲ ਸਾਰੇ ਦੇ ਸਾਰੇ ਆਕ੍ਰਿਤਕ ਮਾਪਦੰਡਾਂ ਵਿੱਚ ਅਰਥਪੂਰਨ ਕਮੀ ਆਈ। ਚੋਣ ਸੂਚਕਾਂਕ, MTSI ਅਤੇ ਪਾਣੀ ਦੇ ਭਰਾਅ ਦੇ ਨੌਂ ਦਿਨਾਂ ਮਗਰੋਂ ਪੌਦੇ ਦੇ ਬਚੇ ਰਹਿਣ ਦੇ ਆਂਕੜਿਆਂ ਦੇ ਅਧਾਰ ਤੇ EML 153, SE 503, EML 125, AVL13119, VL 1010764, I 168, I 204, AVL13056, PML 368, LM 17, *Z. mays spp parviglumis*, PML 373, PML 387, EML 285, JCY 20, I 170, I 185, I 172, PML 1228, PML 243, PML 28, EML 124, LM 6, I 185 ਅਤੇ I 192 ਲਾਈਨਾਂ ਪਾਣੀ ਦੇ ਭਰਾਅ ਦਾ ਟਾਕਰਾ ਕਰਨ ਦੇ ਸਮਰੱਥਾ ਪਾਈਆਂ ਗਈਆਂ। ਪ੍ਰਤੀਰੋਧਕ ਲਾਈਨਾਂ ਦੀ WTC 1 ਦੇ ਨੇੜੇ ਜਾ ਇਸ ਤੋਂ ਵਧੇਰੇ ਸੀ। ਸੰਵੇਦਨਸ਼ੀਲ ਲਾਈਨਾਂ ਲਈ ਬ੍ਰੋਡੇਂਡ ਐਂਥੇਸਿਸ ਸਿਲਕਿੰਗ ਅੰਤਰਾਲ >5 ਦਿਨ ਸੀ ਜਦੋਂਕਿ ਪ੍ਰਤੀਰੋਧਕ ਸਹਿਣਸ਼ੀਲ ਲਾਈਨਾਂ ਲਈ ਇਹ 3-5 ਦਿਨ ਸੀ। ਝੋਨੇ ਵਿੱਚ ਪ੍ਰਤੀਰੋਧਕਤਾ ਲਈ ਜ਼ਿੰਮੇਵਾਰ ਔਰਥੋਲੋਗ ਜੀਨਾਂ ਦੀ ਖੋਜ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ *SNORKEL* ਲਈ ਕੋਈ ਵੀ ਅਰਥਪੂਰਨ ਖੇਤਰ ਨਹੀਂ ਸੀ ਪਰ *Sub1A* ਲਈ ਤਿੰਨ ਵਿੱਚੋਂ ਦੋ ਕੋਡਿੰਗ ਅਨੁਕ੍ਰਮਣਾਂ ਲਈ ਅਰਥਪੂਰਨ ਅਨੁਕ੍ਰਣਮ ਸਮਾਨਤਾ ਸੀ। ਜੀਨ ਸਪੇਸੇਫਿਕ ਪ੍ਰਾਈਮਰਾਂ ਦੇ ਸਿੰਥੇਸਿਸ ਅਤੇ ਐਲੀਲਿਕ ਸਰਵੇਖਣ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਹਨਾਂ ਜੀਨੋਟਾਈਪਾਂ ਵਿੱਚ ਅਰਥਪੂਰਨ ਐਲੀਲਿਕ ਵਿਭਿੰਨਤਾ ਨਹੀਂ ਸੀ। ਜੀਨ ਐਕਸਪ੍ਰੇਸ਼ਨ ਅਧਿਐਨ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਪਾਣੀ ਦੇ ਭਰਾਅ ਦੇ ਸ਼ੁਰੂਆਤੀ ਪੜਾਅ (3 ਦਿਨ) ਉਪਰ ਸਹਿਣਸ਼ੀਲ ਲਾਈਨਾਂ ਵਿੱਚ (15 ਗੁਣਾ ਤੱਕ) ਅਰਥਪੂਰਨ ਫੋਲਡ ਐਕਸਪ੍ਰੇਸ਼ਨ ਸੀ ਜਿਸ ਤੋਂ ਐਨਾਰੋਬਿਕ ਵਿਕਾਸ ਦੇ ਹਲਾਤ ਵਿਰੁੱਧ ਸਹਿਣਸ਼ੀਲਤਾ ਵਿਕਸਤ ਕਰਨ ਵਿੱਚ ਉਹਨਾਂ ਦੀ ਭੂਮਿਕਾ ਦਾ ਪਤਾ ਚੱਲਿਆ। ਜੰਨਸੰਖਿਆ ਸੰਚਰਨਾ ਮੁਲਾਂਕਣ ਤੋਂ ਉਪਲਬਧ ਜਰਮਪਲਾਜ਼ਮ ਵਿੱਚ ਤਿੰਨ ਉਪ-ਜੰਨਸੰਖਿਆਵਾਂ ਦੀ ਹੋਂਦ ਦੀ ਪੁਸ਼ਟੀ ਹੋਈ। ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਭਿੰਨਤਾ ਅਧਿਐਨ ਲਈ ਵਰਤੇ ਗਏ SSR ਮਾਰਕਰਾਂ ਲਈ ਔਸਤਨ PIC ਮਿਕਦਾਰ 0.503 ਸੀ। MTA ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ umc-1021 ਅਤੇ umc-2340 ਨਾਮਕ ਦੋ ਮਾਰਕਰ ਜੜ੍ਹ ਖੇਤਰ ($-\log_{10}$p of 3.91) ਅਤੇ ਜੜ੍ਹ ਆਯਤਨ ($-\log_{10}$p of 11.34) ਨਾਲ ਅਰਥਪੂਰਨ ਤੌਰ ਤੇ ਸਬੰਧਤ ਸਨ।

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CHAPTER I

INTRODUCTION

Maize (*Zea mays* L.) is an annual grass belonging to the tribe Maydeae of *Poaceae* (*Gramineae)*, the grass family. Morphologically maize is a tall grassy C_4 plant with determinate growth habit with height generally varying from one to four meters producing large, green, narrow leaves arranged alternatively along the entire length of solid stem. Being a monoecious plant, the sexes are partitioned into two distinct levels arranged at different plant position. The female flower (pistillate), known as cobs or ears, are produced at fused lateral branch called shank arising from leaf axils, while the male flower (staminate), known as tassel, are at the upper apex of the stem and terminates the main shoot. Protandrous type of flowering habit is orchestrated by maize, where the male flower matures two to three days prior to female flower, which is a prime reason for the predominantly cross pollination type of mating habit. Study from several archaeologists and data from molecular studies suggested domestication of teosinte (*Zea mays ssp. parviglumis*) around 6600- 9000 years ago in the Southern Mexico has given rise to modern days maize plant (Van Heerwaarden *et al* 2011).

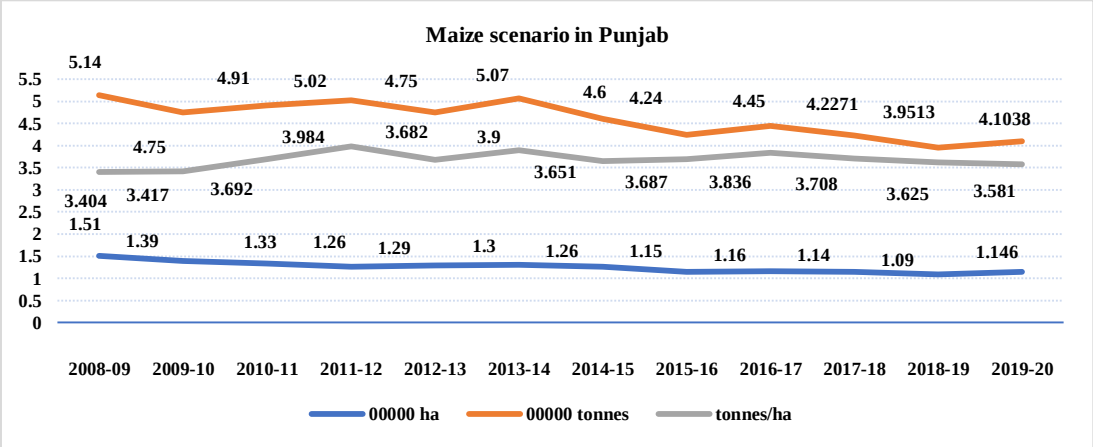
Maize has diverse uses and majority of its share is used for animal feed (around 61%), around 17% of the produce is used as food mostly in the developing countries and around 22% as industrial inputs (Grote *et al* 2021). Maize is industrially important raw product for the production of myriad products such as starch, biofuel, beverages, oils and so on.

Attributed to its highest genetic yield potential amongst all cereals, maize is undoubtedly Queen of cereals being an important source of carbohydrates in dietary among developing countries and as a main feed to the livestock in developed countries. Endowed with wide adaptability habit, maize is grown in almost every corner of the world encompassing more than 165 countries and thriving in almost every edaphic and climatic conditions. Globally maize is an important cereal ranking second in the world in terms of acreage, cultivated on nearly 197.20 million hectares with global production of approximately 1148.48 million metric tonnes in 2018-19 (FAOSTAT 2020).

Globally India ranks fourth in area having 9.02 million ha and seventh in production with 27.71 million tonnes (FAOSTAT 2020). Within India, maize is cultivated across every states except for some Union Territories including, Andaman and Nicobar Islands, Goa, Dadra and Nagar Haveli, and Delhi. Karnataka with an area of 1.42 million ha under maize cultivation producing 4.25 million tonnes stands as the largest maize producing state of the country. Out of total 5033 thousands ha geographical area of Punjab, 4117 thousands ha is

under crop cultivation in the state. The major share of this cultivation land is under the rice and wheat. Only a mere 1.29 lakh ha is under the maize cultivation (Department of Food Processing Punjab 2013)

Detailed data on production, productivity and area under maize cultivation in Punjab over last 12 years is illustrated in the Fig. 1.1.



Source : Ministry of Agriculture & Farmers Welfare, Govt. of India.

Fig. 1.1: Area ('00000 ha), Production ('00000 metric tonnes) and Productivity (tonnes/ha) of maize in Punjab from 2008-09 to 2019-20.

Maize is extensively grown over an array of agroclimatic regions resulting the crop being susceptible to several biotic and abiotic stresses affecting the production. Among the several abiotic stresses, waterlogged condition leading to excessive soil moisture is a major constraint of maize production mostly in the tropical Asian regions (Lone and Warsi 2009). Alterations brought about in the soil properties by flooding create an adverse situation for plant survival (Dat *et al* 2004). Globally around 10% of the land area is affected by waterlogging and it mainly hampers the crop survival by restricting the gaseous exchange leading to hypoxia and anoxia condition and causing poisoning of mineral nutrients and microelements (Settler and Waters 2003). Waterlogging may cause the yield penalty of 15 to 80% depending upon plant genotype, duration, growth conditions and soil properties (Zhou 2010). In India following drought, waterlogging is the second most severe constraint for maize production affecting about 8.50 m ha of arable land. Twenty-five lakh ha out of eighty lakh ha of maize production is exposed to waterlogging causing an average yield loss of 25-30% almost every year (DMR 2001). As per the report from Punjab Government, waterlogging is exposed to an area of 2 lakh ha of fertile arable land of the state. With the changing climatic conditions, the rainfall patterns have been erratic and there has been increased frequency of several events such as drought, flooding. These events have further worsen the scenario.

Maize is grown throughout the year but, *kharif (*June -October) sown maize is the main season, generally characterized by heavy monsoon leading to water stagnation at one or other crop growth stages, especially in Indo-Gangetic Plains and North-Eastern region. The rate of diffusion of gases under such soil saturation is 10^4 slower when compared to normal soil water condition (Armstrong 1979).

Accumulation and translocation of toxic substances like ethanol, resulted from anaerobic respiration, on the upper plant surfaces through transpiration pull leads to necrosis of leaves and wilting. Apart from intrinsic toxicity, several extrinsic toxic heavy metal ions get accumulated as the reduction potential of the soil changes and these ions enter the soil-water solution. Iron and manganese compounds gets converted into free ions under high reduction potential.

When the flood water recedes and the plants are re-exposed to normal oxygenated environment, plants get post- anoxic injuries which may be characterized by softening of tissues and gradual drying of leaves leading to death of tissues (Gutteridge and Halliwell 1990). Superoxide radicals, iron induced hydroxyl radicals and other reactive oxygen species (ROS) inducing membrane damage are assumed to be major culprit for this post-anoxic damage.

Under soil saturation, the concentration of dissolved oxygen in soil water falls drastically, from 230 mmol m^{-3} to 50 mmol m^{-3} hindering the ATP synthesis in root tissue leading to energy deficient condition (Grichko and Glick 2001). Since the energy generation in the root and ion transport are linked to each other, the root tissues are highly sensitive to oxygen supply (Drew 1988). Under anaerobic condition, the energy metabolism is not at a level that will drive active transport through H^+ translocating ATPase in plasma membrane (Armstrong and Drew 2002). Under hypoxic condition led by waterlogging, oxygen which is the terminal electron acceptor in the mitochondrial electron transport system gets depleted leading to the limitation in production of ATP and thus declining the ratio of ATP : ADP (Drew 1997). To alleviate the damage due to depleted energy in the plant system, plants shift its normal mitochondrial aerobic energy production mechanism into anaerobic fermentation to yield ATP and $NADPH^+H^+$. When the production of ATP through oxidative phosphorylation gets inhibited as a result of limited cytochrome oxidase activity under anoxic condition and fermentative pathway is opted out, cellular metabolism and function is impaired as the efficiency of ATP generation is sharply lowered (Ricard *et al* 1994a, Geigenberger 2003).

Compared to respiration of one molecule of hexose producing up to 39 molecules of ATP, fermentation of such molecule yields a maximum of mere three molecules of ATP. Furthermore, ionic balance gets disturbed leading to energy depletion and membarane

deplorization (Buwalda *et al* 1988). Hence, the survival of plants under hypoxic and anoxic condition is mostly determined by how efficiently a plant execute its alternate survival strategy such as shift in respiration, some physiological other morphological amendments (Chirkova *et al* 1984). As a normal pathways of respiration is deviated, the anaerobic proteins gets upregulated to smoothen the altered pathways (Ho and Sachs 1989). These proteins are known as anaerobic polypeptides (ANPs) and are comprised of glycolytic and fermentation enzymes such as alcohol dehydrogenases (ADH) (Mujer *et al* 1993, Liao and Lin 1995).

Another important morphological adaptation is formation of aerenchyma, which are the hollow air chambers that facilitates the diffusion of oxygen, accumulated from the exposed upper surface, from shoots to roots. Aerenchyma formation occurs either by any one of two mechanisms, lysigeny or schizogeny, or by the combination of both (Kawase 1981). Waterlogging causes detrimental effect in normal plant functioning and lead to several adversities such as wilting of leaves, chlorosis, reduction in photosynthate accumulation, cell membrane disruption, hinderance in normal mineral and nutrients uptake and altered physiological pathways, ultimately leading to poor plant stand and prolonged stagnation of water can even leads to complete crop failure. This stress tends to alter the soil profile, modify the soil redox potential affecting the availability and uptake of essential nutrients. Since the energy production through glycolytic pathways, mainly ethanolic and lactic acid fermentation are limited and most of the energy is directed by the plants in the movement of solutes involved in pH regulation and transport of sugars involved in anaerobic catabolism reactions, the energy required for the uptake and transport of nutrient ions such as N, P, K and mineral ions gets substantially reduced. (Pang *et al* 2007, Gibbs and Greenway 2003, Smethurst *et al* 2005). Availability of Nitrogen is severely affected as most of the available nitrogen is lost through the process of denitrification, leaching and a small amount through volatilization (Srivastava *et al* 2010). In the absence of free oxygen under anoxic condition, soil microbes use nitrate (NO_3) as an alternative electron acceptor and is reduced to nitrite (NO_2), several nitrous oxides like N_2O, NO and molecular nitrogen (N_2) (Gambrell *et al* 1991). Reactive oxygen species (ROS) formation and scavenging activity are in homeostasis under normal growth conditions.

But under waterlogging, highly induced intracellular environment and saturated electron transport components lead to explosion in reactive oxygen species (ROS) which is infamous for causing enzyme inactivation, membrane deterioration, oxidative injury to nucleic acids and lipid peroxidation (Shewfelt and Purvis 1995). Several scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), polyphenol oxidase (PPO) and other non- enzymatic compounds such as ascorbate, glutathione, tocopherols and

other phenolic compounds continuously function to maintain the homeostasis, but as the production of the ROS exceeds the scavenging the plants suffer oxidative stress. (Alscher *et al* 1997, Noctor and Foyer 1998).

Identification of ortholog genes through insilico approaches and their characterization help in isolating putative genes contributing to different traits. Several maize orthologs of rice and vice versa have been studied and genes have been established for several characters. Maize ortholog of rice *GS5* has been identified for controlling kernel development in maize (Liu *et al* 2015). Another maize ortholog of rice *GW2* responsible for kernel size and weight has been elucidated (Li *et al* 2010). Rice ortholog of maize *INDETERMINATE1* gene has been identified for promoting flowering in rice. In rice, *Sub1A* gene confers tolerance against long term submergence and identification of ortholog region in maize for *Sub1* helps in validating the involvement of Sub1A in conferring tolerance against waterlogging in maize. With the cheaper sequencing techniques and reliable and quicker genotyping approaches, molecular screening has taken over the conventional field screening. Genotyping with molecular markers allows a precise, objective, and rapid cultivar identification, which has been proven to be an efficient tool for crop germplasm characterization, collection, and management.

In Punjab the crop is sown in the month of June which coincides with the monsoon season. If there is waterlogging, it adversely affects the germination, plant growth and ultimately the yield. The waterlogged condition of a particular area cannot be managed but we can identify those lines that can tolerate such saturated condition to avoid the losses resulting from excessive soil moisture. The plant undergoes several modifications in physiological, morphological, biochemical, anatomical and molecular levels to withstand against such stressful condition. Identification of such candidate genes associated with these alterations will contribute to maize breeding programmes in development of more stress-tolerant lines. Though waterlogging is the major abiotic stress for this water-sensitive crop, proper study on characterization of important inbred lines sown in this region has not been done. Various genetic and molecular components infer tolerance to crop, but in absence of proper study on the allelic variation for the identified genes enough information is not available. Thus, to elucidate all those gaps the study was conducted with following objectives:

i. To screen maize inbred lines for waterlogging tolerance.
ii. Identification of orthologs in maize for waterlogging tolerance.
iii. Screening for allelic variation in inbred lines for identified genes involved in waterlogging tolerance in maize.

CHAPTER II

REVIEW OF LITERATURE

Maize is extensively grown over an array of agro-climatic region resulting the crop being susceptible to several biotic and abiotic stresses affecting the production leading to the yield penalty of 15 to 80% depending upon the genotype, duration, growth conditions and soil properties. Waterlogging stress severely affect the normal growth and productivity and has been the area of interest requiring the immediate attention to attain the mission of food security. Stagnation of water leading to excessive soil moisture led by superfluous precipitation, unscientific irrigation and poor outlet of water or erratic rainfall pattern causes waterlogging, causing the exclusion of soil air leading to anoxic soil environment.

The review pertaining to this research problem is discussed below:

2.1 Waterlogging stress, adaptation mechanism and molecular response

2.1.1 Modifications against waterlogging stress: Physiological, morphological, biochemical, anatomical and molecular modifications.

On response to the water logging leading to the hypoxia and anoxia conditions, plants brought about several modifications at various metabolic processes and changes at physiological, morphological, biochemical, anatomical and molecular levels as these conditions greatly impaired the plant life (Kennedy *et al* 1992, Perata and Alpi 1993, Ricard *et al* 1994b, Drew 1997).

Yan *et al* (1995) flooding treatment to potted maize plants observed several modifications in corn leaves that included elevated chlorophyll breakdown, peroxidation of lipids, enhanced membrane permeability and production of superoxide and hydrogen peroxide with older leaves being highly affected. These modifications can be linked with the reduced activity of superoxide dismutase (SOD) under excess water stress condition.

Lizaso and Ritchie (1997) reported that the root zone saturation severely impacted kernel number per ear leading to the reduced yield and had a major effect on other aspects of plant growth that includes curtailed expansion of the photosynthetic areas reducing the photosynthesis and reduced green leaf area due to increased leaf senescence. The fall in carbohydrate (CHO) concentration under root zone saturation might be associated with the leakage loss brought about by the partial loss of membrane integrity along with increased utilization of previously accumulated CHO with reduced assimilation. Though both early and late saturation has similar responses, doubling the saturation period further reduced the overall biomass by an additional 10%.

Lone and Warsi (2009) studied the impact of excess soil moisture at seedling stages as they being very susceptible stages. The susceptible genotypes showed poor germination and plant stand. Some of them did not germinate at all and others did not reach two-leaf stage. Waterlogging at knee height stage resulted in immediate wilting of the plants starting from the base while in case of the leaves wilting started from the tip and moved towards their base and leading to lodging. While in tolerant genotypes adventitious roots were profusely developed appearing up to five nodes. In response to the waterlogged condition, Anthesis Silking Interval (ASI) widened (>8 days) which severely affected the pollination and seed set ultimately reducing the overall production. Along with reduced plant and ear height, several physiological attributes also got affected like decreased transpiration rate and SAPD values (relative greenness) and increased Photosynthetically Active Radiation (PAR).

Yong-zhong *et al* (2010) subjected twenty inbred lines to artificial flooding condition at seedling stages to investigate and identify the best criteria for the selection of waterlogging tolerant genotype. Waterlogging tolerance coefficient (WTC) was employed to identify tolerant genotype along with identification of most susceptible stage which showed V_2 (second leaf stage) to be the most susceptible one and 6 days after flooding to be the best continuous treatment time at which there was a maximum reduction in the dry weight of both shoots and roots compared to control. Peroxidase (POD) activities and malondialdehyde (MDA) contents were measured and found to be negatively and significantly correlated with correlation coefficient being -0.9686 (P<0.0001) which could be used as a reference index for material screening along with other parameters like leaf chlorosis and WTC of shoot dry weight.

Zaidi *et al* (2004) standardized the screening method (cup method) and identified maize genotypes tolerant to excess moisture stress. Adaptive responses to the stress were also studied. V_2 i.e. early seedling followed by V_7 i.e. knee-height stage were found to be the most susceptible among the four stages considered in the experiment i.e. V_2, V_7, VT (tasseling) and R_1 (milk stage). Stress leads to poor seed set and ultimately resulted in poor production brought about by affecting several growth and biochemical parameters and weakened anthesis and silking. The genotypes tolerant to such hypoxia/anoxic condition are found to be associated with stem tissues having a good accumulation of carbohydrate, moderate stomatal conductance, <5 days ASI, ability to develop the brace root at the earliest and root with high porosity.

To decipher the morpho-physio-biochemical and molecular responses of maize under waterlogging, Mahmood *et al* (2021) carried out the study using four hybrid of maize grown under the waterlogging, salinity and combination of both. The study revealed that combined

action of waterlogging and salinity caused higher ROS production and higher chlorophyll reduction when compared to sole stress. Similarly the enzymatic activities of CAT (catalase), SOD, POD and APX (ascorbate peroxidase) were also significantly enhanced. Morphological parameters like root-shoot length, their fresh and dry weight, leaf width, leaf number per plant and chlorophyll and carotenoid content were also significantly reduced.

Mano and Nakazono (2021) thoroughly reviewed the various root traits under the genetic regulation to impart tolerance against severe water stagnation and discussed the associated bottlenecks making development of waterlogging a cumbersome task. Non-availability of appropriate donors and the polygenic nature of the trait with complex genetic factors associated were considered to cause major setbacks. Identification of appropriate flooding-tolerance-related traits with establishment of accurate evaluation method for the test of these traits, identification of major QTLs using diverse set of mapping population like near-isogenic lines (NILs), introgression lines (ILs), chromosome segment substitution lines (CSSLs) to identify the chromosomal region and further pinpointing by fine mapping and transfer of these identified genes via several pyramiding approaches could help develop genotypes with desired level of tolerance against abiotic stresses.

2.1.2 Quantitative Trait Loci (QTLs) associated to waterlogging in maize.

Qiu *et al* (2007) carried out two experiments, *viz.* I and II and identified twenty-five and thirty-four QTL respectively, associated with tolerance to waterlogging tolerance with their effect ranging between 3.9 to 37.3%. Major tolerance QTL associated with shoot, root and total dry weight, plant height, and WTC were mapped on chromosome 4 and 9 and consistently detected in both the experiments. Other secondary QTLs were mapped on chromosome 1, 2, 3, 6, 7 and 10 which were trait and environment-specific.

Yu *et al* (2019a) reported that Group VII ethylene response factors (ERFVIIs) which play important roles in ethylene signaling and plant responses to waterlogged conditions to be absent in maize. Instead, *ZmEREB180* was found to be tightly associated in maize against such stresses identified by a candidate gene association analysis of the *ZmERFVII* gene family. This waterlogging-responsive gene, *ZmEREB180*, expression responded to flooding and regulated by ethylene. Variations in the 5′-untranslated region (5′-UTR) and copious amount of produced mRNA produced through overexpression was linked with survival rate under waterlogged condition through profuse development of adventitious roots and regulation of antioxidants level. Transcriptomic assays further provided the evidence of participation of *ZmEREB180* in the regulation of adventitious root formation and reactive oxygen species (ROS) homeostasis which concluded the involvement of *ZmERFVII* gene in waterlogging tolerance.

Campbell *et al* (2015) screened a diverse set of maize nested associated mapping (NAM) founder lines and identify highly tolerant and sensitive lines where these lines were subjected to transcriptome analysis. Tolerant lines when compared to sensitive lines demonstrated late senescence and lower oxidative stress levels. Transcriptome analysis showed the abundance amount of several fermentation associated genes and an unannotated Pyrophosphate-Dependent Fructose-6-Phosphate 1-Phosphotransferase gene during submergence in tolerant lines compared to sensitive lines. A co-expression network enriched for CBF (C-repeat/DRE binding factor: C-repeat/DRE binding factor) genes were activated on submergence in all the lines but with the varying degree; increased level in tolerant lines. Screening of the RIL population identified a major QTL named Subtol6 as a major submergence tolerance locus located in chromosome 6. Two candidate genes *HEMOGLOBIN2* and *RAV1* underlying Subtol6 based on contrasting expression patterns were identified.

Mano *et al* (2005) identified QTLs for adventitious root formation on chromosomes 3 (bin 3.07-8), 7 (bin 7.04-5) and 8 (bin 8.05) in maize.

Mano *et al* (2007) identified QTLs for aerenchyma formation under non-Xooding conditions were located to two regions of chromosome 1 (identified as *Qaer1.02-3* and *Qaer1.07*), chromosome 5 (*Qaer5.09*), chromosome 8 (*Qaer8.06-7*), chromosomes 1 (*Qaer1.01*) and 5 (*Qaer5.01*) in maize.

Osman *et al* (2013) QTLs associated with plant height, root length, root dry weight, shoot dry weight and total dry weight were identified. Six QTLs (ph6-1, rl1-2, sdw4-1, sdw7-1, tdw4-1 and tdw7-1) were identified at two consistent stages of seedling development.

Liu *et al* (2012) reported hypoxia conditions mediated by waterlogging switched respiration from aerobic to anaerobic fermentation resulting in alterations in gene transcription and synthesis of proteins. A quantitative real time PCR (qRT-PCR) assay was employed to determine the expression of miRNAs to characterize the involvement of those miRNAs and their targets in response to hypoxia condition. Several miRNAs *viz*. miR159, miR164, miR167, miR393, miR408, and miR528 were found to be key regulators in the post-transcriptional regulatory mechanisms which were mainly involved in root formation and responses to waterlogged condition. These results suggested miRNAs to be active participants in the signal transduction at the hypoxia condition.

Yu *et al* (2019b) analyzed the genetic polymorphism using two lines that had contrasting phenotypes in response to waterlogging. A novel QTL associated with root related traits, qWT5.04, was mapped and the key candidate gene, *GRMZM2G179981*, within that target QTL showed differential expression between two lines at the transcriptional and

proteomic level. Among the thirty-seven variations that were located within the promoter region (1.5-kb) of *GRMZM2G179981*, thirteen variants were found to be significantly associated with root related phenotypes. These findings demonstrated complete linkage disequilibrium and overlapped with variations in those contrasting lines suggesting the haplotype to be the causal functional locus. Reduced level of leaf injury under flooding condition strongly proposed *GRMZM2G179981* to be the major gene for tolerance against waterlogging.

To elucidate the chromosomal location of gene responsible for barrier to ROL, Watanabe *et al* (2017) developed a series of introgression lines containing different segments of *Z. nicaraguensis* in the genetic background of Mi29. Among 45 ILs developed, three ILs (IL#17, IL#20 and IL#40) did not produce seeds to validate their involvement, while the study from the remaining 42 ILs deciphered three ILs, namely IL#11, IL#5 and IL#12 and only IL#11 was formed at tight ROL barrier. The locus was validated by the phenotypic results coupled with DNA markers revealing their position to be within around 22.6 Mb on the short arm of chromosome 3 between two SSR markers bnlg1325 and bnlg1113.

Mano *et al* (2012) constructed intraspecific linkage maps for root aerenchyma formation by studying four segregating population of *Z. nicaraguensis*. 209 SSR markers distributed across the entire maize genome along with an additional 27 markers mapped to bins 1.05-1.07 were genotypes and polymorphic markers were employed for the construction of linkage maps. CIM analysis identified two QTLs, one each in chromosome 1 (Qaer 1.06-1.07) and chromosome 7 (Qaer7.01) together explaining 32% of total phenotypic variation. A chromosomal rearrangement caused by paracentric inversion was revealed by the orders of the markers from the bins 4.08-4.11 on the long arm of chromosome 4 and further critical investigation revealed the position of breakpoint to be between two markers, namely umc1847 (bin4.07) and phi066 (bin4.08).

Mano and Omari (2013) from the set of 45 ILs developed by introgression of different fragments of *Z. nicaraguensis* on genetic background of Mi29 identified a line IL#18 containing a segment from the long arm of chromosome 4 introgressed from *Z. nicaraguensis* to be highly waterlogging tolerant suggesting a major QLT responsible for flooding tolerance to be localized in long arm of chromosome 4 of *Z. nicaraguensis*. Apart from this, portion from chromosome 2 also orchestrated similar tolerance ability but the data required further validation. Similarly IL#4 possessed a fragment from chromosome 1 (bin1.03-1.10) which showed significant aerenchyma formation. The region overlapped the earlier reported region QTL Qaer1.06-1.07 which further validated the results given by the same workers in 2013.

An introgression line AE24-50-44-91 (IL-AE91) was constituted by introgressing four constitutive aerenchyma formation (CAF) QTLs namely, Qaer1.06-1.07, Qaer1.11, Qaer5.09n and Qaer8.05 in genetic background of Mi29 from *Z. nicaraguensis* and was studied to prove that constitutive aerenchyma formation in root is an important mechanism for conferring tolerance in plants. This study conducted by Gong *et al* (2019) showed significant improvement in aerenchyma formation which were 4.6%, 8.5% and 7.1% respectively when compared to Mi29 which were mere 2.2%, 3.8% and 3.1% respectively. And since the introgression line was more tolerant than the inbred line Mi29, the study revealed the association of CAF to impart tolerance against waterlogging.

2.1.3 QTLs associated to waterlogging in rice and related crops

Xu and Mackill (1996) identified the gene conferring waterlogging tolerance in rice. Random-amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) were employed to map submergence tolerance in plants obtained from a cross between IR40931-26 (tolerant indica line where tolerance gene has been transferred from FR13A) and PI543851 (susceptible japonica line). Those markers were mapped to a region of chromosome 9. A submergence tolerance QTL, *Sub1*, was situated 4cM from the RFLP marker C1232 which accounted for 69% phenotypic variance.

Xu *et al* (2006) described the identification of a cluster of three genes at the *Sub1* locus *viz. Sub1A, Sub1B* and *Sub1C*. Among these three, *Sub1B* and *Sub1C* are found in the *Sub1* region of all rice accession analyzed while the presence of Sub1A is variable. Two alleles of *Sub1A, Sub1A-1*(tolerance-specific allele) and *Sub1A-2* (intolerance specific allele) were identified. *Sub1A-1* which brought about enhanced tolerance in plants, downregulation of *Sub1C* and upregulation of Alcohol dehydrogenase 1 (Adh1) indicated it to be the major factor of submergence tolerance.

Hattori *et al* (2009) identified the genes *SNORKEL1* and *SNORKEL2* responsible for the ability of the plant to appreciably elongate its internodes, which have snorkels like hollow structure to allow gas exchange and preventing drowning of plants. These genes under deep water condition are triggered by encoding ethylene response factors involved ethylene signaling. Thus accumulated ethylene induce the expression of these two genes whose products then triggered significant internode elongation via gibberellin.

Angaji *et al* (2010) selected Khao Hlan On, tolerant rice genotype to map QTLs associated with flood tolerance using IR64 as a recurrent parent. 1475.7 cM long linkage map was constructed with the average interval of 11.9 cM using 135 polymorphic SSRs and 1 indel marker. Five putative QTLs were reported that includes qAG-1-2 (chromosome 1), qAG-3-1 (chromosome 3), qAG-7-2 (chromosome 7) and, qAG-9-1 and qAG-9-2

(chromosome 9) demonstrating 17.9 to 33.5% of phenotypic variation and with LOD scores of 5.69-20.34.

Kurokawa *et al* (2018) identified Leaf Gas Film 1 (LGF1) gene responsible for the determination of leaf gas films using a rice mutant drp7 which lacked the gas film and its wild type (Kinmaze). Leaf gas films on hydrophobic leaves enhanced the gas exchange underwater. LGF1 gene regulates C30 primary alcohol synthesis required for production of sufficient epicuticular wax platelets, leaf hydrophobicity and gas films on under water leaves which ultimately enhanced under water photosynthesis by 8.2 folds and contributing to submergence tolerance.

Sequence variation survey of *Sub1A*, *Sub1B* and *Sub1C* genes for 179 cultivars grown in waterlog prone sites was demonstrated by Singh *et al* (2020). They performed the sequence survey in Indica (17) and Japonica (4) cultivars, *O. rufipogon*'s cultivated accessions (14) and wild accessions (3). Marker survey for *Sub1A* and *Sub1C* was performed for 109 genotypes, 76 cultivars and 160 varieties (using CAPS markers). The result identified eight SNP haplotypes for *Sub1A* gene, nine SNP haplotypes for Sub1B gene and 67 SNP haplotypes for *Sub1C* gene based on seven, seven and 38 SNPs/InDels identified in *Sub1A*, *Sub1B* and *Sub1C* genes respectively. Due to the availability of high level of variation in *Sub1C* gene indicated by 29 exonic regions as compared to *Sub1A* (2 exonic regions) and *Sub1B* (one exonic region), *Sub1C* was referred to be of archaic origin and both *Sub1A* and *Sub1B* were hypothesized to have been originated from *Sub1C* by the action of duplication and divergence. New alleles of all three *Sub1* genes were elucidated and tolerant lines showing both *Sub1A-1* dependent and *Sub1A-1* independent tolerance (tolerance was exhibited though they were either null for *Sub1A* gene or tolerance was exhibited though submergence sensitive *Sub1A-2* allele was present) were identified opening the new avenues for the researchers to explore and identify other sources of tolerance that is not governed by *Sub1A* locus.

An in-depth study on gene expression in root tissue under waterlogging condition was carried out in Barley by Borrego-Banjumea *et al* (2020) using two waterlogging tolerant barley genotypes, namely Yerong and Deder2. RNA-sequencing revealed 6736 DEGs in roots of Yerong at 72 hours of flooding, 5482 DEGs in Deder2 at 72 hours and 4538 in 120 hours of flooding. Several energy related genes such as β-fructofuranosidase and invertase were downregulated while SUS, SPS, phosphofructokinase and fructose-bisphospahte aldolase genes expression were induced. Genes associated with nitrogen and amino acid metabolism, AS (asparagine synthetase), GS (glutamate synthetase), GOGAT (glutamate synthase) and nitrogen transporters were downregulated. Genes involved in ROS scavenging to maintain homeostasis were downregulated. Around 298 transcription factors were identified among

which 173 were downregulated and 125 were upregulated. Majority of TFs belonged to MYB, AP2/EREBP, NAC, WRKY, bHLH, bZIP, HB and G2-like families.

2.1.4 Identification of novel genes and validation through transcriptional and expression analysis.

Kaur *et al* (2021) performed the transcript profiling of Maize at 24-96 hours of waterlogging with 23 anaerobic polypeptides (ANP) and 125 miRNA. Two contrasting maize inbred lines, I110 (susceptible to waterlogging) and I172 (tolerant to waterlogging) were taken to study the transcriptional and anatomical changes in response to waterlogging. In tolerant line, maximum upregulation was seen for alanine aminotransferase (AlaAT) (34.54X), mitogen activated protein kinase (MAPK) (8.11X) and pyruvate kinase (PK) (6.45X), while in susceptible line maximum fold change was seen for sucrose synthase (44.94X), aspartate aminotransferase (AspAT) (25.63X), alcohol dehydrogenase(ADH) (16.56X), NADP dependent malic enzyme (Ndm) (14.22X) and auxin response factor (ARF) (12.3X).

For miRNA, the tolerant line show maximum upregulation for miR168 target (41.64X), mi408 target (36.75X) and miR7a target (20.82X), while in susceptible line, maximum upregulation was for miR408 target (63.12X), miR172 (29.65X) and mi167 targets (25.11X).

Arora *et al* (2017a) studied the structural and functional composition of Cell Wall Modification (CWM) genes along with their expression patterns in maize under flooding condition. Cell wall in tolerant lines gets degraded by the action of reactive oxygen species led programmed cell deaths which leads to the formation of hollow chamber called aerenchyma for diffusion of air between shoot and root. Expression analysis revealed differential expression pattern in susceptible and tolerant lines. Endoglucanases (AC199765.4_FG008) showed 4.6 fold change in tolerant lines while 2.3 fold change in susceptible line. Other CWM genes, Arabinogalactan, XTH, expansins, PAE, and pectin esterases showed varying fold change with upregulated expression in tolerant lines and downregulation in susceptible lines. All the 12 CWM genes, except for arabinogalactan (upregulated in susceptible line), showed upregulated expression in tolerant genotypes when the expression was observed in leaf tissue. One gene, Pectin esterases, showed downregulation in both tolerant and susceptible lines 1.2 fold change for tolerant line but a whooping 73 fold change for susceptible line.

Transcriptome analysis using RNA sequencing of roots of waterlogging tolerant inbred HKl1105 was carried out by Arora *et al* (2017b) revealing 21364 DEGs (Differentially expressed genes) under flooding condition. Different genes responsible for energy production

under anaerobic condition revealed different level of expression. Invertase gene showed 49 fold change, sucrose synthase 7 folds change, hexokinase showed 36-fold change, phosphofructokinase showed 7-fold change, phosphoglycerate dehydrogenase showed 8.8-fold change, and Alcohol dehydrogenase showed 7.6-fold change. For gens responsible for programmed cell deaths and aerenchyma, respiratory burst oxidase homolog (RBOH) was upregulated and showed 12.1-fold change while metallothionein (MT) was downregulated upto 7-folds. Genes encoding expansions, polygalacturonases and xyloglucan endo-transglycosylasesor hydrolases (XTH) were highly upregulated up to 41.8-folds, 16.1 folds and 10.7 folds respectively. Other genes responsible for ethylene synthesis were also significantly upregulated. Two genes namely, *GRMZM2G377341* and *AC197672.3* were upregulated at 1.2 and 2.6 folds respectively, while four genes from ERFVII were upregulated and other four were downregulated. Methionine aminopeptidase (MetAP) showed differential pattern of gene expression with four genes being upregulated and two being downregulated.

To elucidate the expression pattern of three waterlogging sensitive genes, Panozzo *et al* (2019) collected and evaluated 19 commercial maize along with B73 RefGen_V3 and demonstrated a contrasting results as compared to earlier researchers. *CYP81D8* gene was earlier reported to be strongly down-regulated in tolerant lines when subjected to waterlogging, while such clear-cut correlation was not seen by Panozzo and his co-workers. The level of transcript for the gene was varying depending on the hybrids and were on both directions. For the PFP gene whose expression was reported to highly downregulated under waterlogging showed highly upregulated in this experiment. *AOX1A* gene showed the consistent result and showed the same expression pattern as all earlier reports. This gene showed strong downregulation and was considered to the important gene to be studied for investigation of waterlogging. *CYP81D8* gene codes for stress-related cytochrome P450, PFP gene involves in glycolytic reactions and *AOX1A* gene is associated to maintenance of ETC and TCA (Tricarboxylic acid) cycle.

Vitreoscilla hemoglobin (VHb) is a special kind of hemoglobin found in gram-negative aerobic bacterium *Vitreoscilla* that has proven role towards tolerance against anaerobic stress. To study its effect in maize under waterlogging, Du *et al* (2016) transformed this gene in maize via particle bombardment in immature Hi-II zygotic embryos and analyzed for their tolerance against long term flooding. The transgenic maize seedlings exhibited enhanced tolerance when compared to negative control and showed the upregulated expression of alcohol dehydrogenase (ADH1) and POD activity. Overexpression of VHb enhanced the shoot height, root length and number, and their dry matter accumulation when compared to negative control under waterlogging condition.

With an aim to develop stress-resilient maize lines having combined tolerance towards two different quantitative traits, *viz.* waterlogging and drought, Das *et al* (2021), employed RCGS (rapid cycle genomic selection) approach on two multiparent yellow synthetic population, namely MYS-1 and MYS-2 applying GEBVs (Genomic estimated breeding values). Both genomic selection (GS) and phenotypic selection (PS) yielded significant gains, with GS showing greater gains towards drought and PS towards waterlogging. Employment of RCGS approach for the improvement of crops against combined several stresses without any yield penalty under optimal conditions could be a break-through approach and this could be achieved through constitution of multiparent population involving the suitable donors and elite germplasm with broad genetic bases. RCGS is still at its early phase and there is urgent need to develop some suitable mathematical model to predict and select the population at early stages to save the precious resources.

Guo *et al* (2021) employed GWAS (genome wide association study) and BSA (bulked sample analysis) to understand and elucidate various QTLs associated with waterlogging tolerance. They concluded that plant height (PH), Root length (RL), shoot dry weight (SDW) and root dry weight (RDW) are the major traits to be studied for identification tolerant lines. Population structure analysis revealed that the tropical maize can be categorized in two major phylogenetic subgroups. GWAS using 39266 SNPs (Single nucleotide polymorphism) distributed across the whole genome characterized 49 such SNPs associated with the trait and their location was found to be dispersed across the entire 10 chromosomes except for chromosome 10. BSA on the other hand revealed 71 significant SNPs across the entire genome except for chromosome 5. Six bins, comprising one from chromosome 1 (bin1.07), one bin from chromosome 2 (bin2.09) and two bins form chromosome 6 (bin6.04, bin6.01) and chromosome 7 (bin7.02, bin7.03), were identified in both the BSA and GWAS study. Nine potential candidate genes has always been reported.

Pedersen *et al* (2021) studied on the status of barrier to radial oxygen loss formation in lateral roots using Mi29 inbred line, an accession of *Z. nicaraguensis* (CIMMYT 13451) and an introgression line (IL#468) possessing chromosomal segments of *Z. nicaraguensis* in the genetic background of Mi29. They demonstrated that along with the main axis of adventitious roots, lateral roots also can form a barrier against radial oxygen loss and barrier formation was governed by the segment introgression from chromosome 3 of *Zea nicaraguensis* as barrier formation was observed in both *Z. nicaraguensis* and IL#468 but not in Mi29. They also observed that barrier formation was exhibited by only those lateral roots that have emerged from surface of the adventitious roots but those emerging from inner cortical region showed no barrier formation. Similarly while studying the aerenchyma

formation in *Zea nicaraguensis*, it was found that proportion of aerenchyma was 1.3 to 1.4 times higher under waterlogging as compared to control. Similarly among all three plant materials taken under study, *Z. nicaraguensis* showed maximum aerenchyma area and it was around 1.9 to 2.0 folds greater to both.

Mukhtar *et al* (1990) studied the effect of waterlogging for two years by subjecting the maize plants to artificial stress condition at different physiological stages of growth and concluded that regardless the growth stage of the crop, flooding stress reduced the grain yield at all stages under study but the severe reduction was at the early vegetative stage (within first 36 days of sowing) characterized by reduction in plant-canopy height, dry matter accumulation and overall yield.

Dhillon *et al* (1998) evaluated five hybrids along with one composite across different moisture regimes and demonstrated that excess moisture significantly reduced the plant height, increased the leaf yellowing and delayed the silking period leading to broaden ASI. It was also shown that as the duration of flooding was increased, the severity also increased accordingly and after 96 hours continuous flooding, the plants were not able to recover from the stress shock suggesting 72 hours of flooding to be the critical period.

Sivakumar *et al* (2019) carried out an experiment to find the association between the SNP marker and four yield related traits including, number of kernel rows per ear (NKR), number of kernel columns per ear (NKC), 100 grain weight (HGW) and grain yield (GY) using 93 diverse maize accessions. Population structure was estimated using STRUCTURE 2.2 software which revealed 5 subpopulations and the marker trait association study performed using TASSEL 4.0.1 showed a total of 36 MTAs between markers and traits.

With an aim to identify the SNPs associated with the yield and saccharification efficiency of maize stover, a GWAS study was conducted by López-Malvar *et al* (2021) using 408 RILs generated from a MAGIC population; founder being EP17, EP43, EP53, EP86, PB130, F473, A509, and EP125. BLUE (Best Linear Unbiased Estimates) data was generated combining the data over years and association between the traits and markers was elucidated using FarmCPU based on MLM model using R. The study identified 13 SNPs to be significantly associated with increased stover yield and 2 SNPs to be significantly associated with improved saccharification efficiency.

CHAPTER III

MATERIAL AND METHODS:

The present study entitled "**IDENTIFICATION of MAIZE GENOTYPES TOLERANT to WATERLOGGING and THEIR CHARACTERIZATION USING RICE ORTHOLOGS**" was carried out in the laboratories of School of Agricultural Biotechnology and maize field experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during the years 2019-20 and 2020-21.

3.1 Plant materials

Plant material employed for the experiments was procured from the Maize Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. A set of genetically diverse maize 119 inbred lines belonging to different heterotic pools along with one accession of teosinte (*Zea mays spp parviglumis*) was used to conduct the experiments. Inbred lines used in the current study is presented in Table 3.1.

Table 3.1: List of genotypes used in experiment

AVL13056	EML 240	I 177	LM 11	PML 82	PML 1241
AVL13119	EML 272	I 179	LM 12	PML 169	SE 503
AVM11207	EML 279	I 182	LM 13	PML 224	SE 533A
CHT 219301	EML 282	I 183	LM 14	PML 243	SE 544
CM 139	EML 283	I 185	LM 16	PML 333	SE 547
CM 140	EML 285	I 187	LM 16 Zp-1	PML 368	SE 549
CML 32	I 105A	I 188	LM 17	PML 373	SE 557
CML 451	I 108	I 189	LM 19	PML 384	SE 563
EL 1	I 121	I 190	LM 20	PML 387	SE 567
EL 2	I 132	I 192	LM 21	PML 400	SE 571
EML 52	I 161A	I 200	LM 22	PML 503	SE 601
EML 53	I 162	I 202	LM 23	PML 898	SE 603
EML 57	I 164	I 204	LM 24	PML 920	SE 604
EML 122	I 165	I 205	LM 26	PML 1012	SE 607
EML 124	I 168	I 206	NL 219605	PML 1224	SE 610
EML 125	I 169	I 230	NL 219608	PML 1225	SE 613
EML 126	I 170	I 231	PAC 753	PML 1228	SE 616
EML 128	I 171	JCY 20	PB Sathi	PML 1229	SE 617
EML 130	I 172	LM 5	PML 28	PML 1230	VL-1010764
EML 132	I 173	LM 6	PML 41	PML 1231	*Z. mays spp parviglumis*

3.2 Cup Experiment

This study was carried out in the growth chamber facility available at Department of Plant Breeding and Genetics, Punjab Agricultural University. Soil collected from the experimental area was sieved to get clod free soil and mixed finely with vermi compost at a 4:1 ratio. Basal fertilizer dose is mixed into the soil based on soil weight (taking weight of soil in 1 hectare to be 2200 metric tons). After thoroughly mixing, soil was filled in each 250 ml plastic cup such that the soil remains around 3 cm below the rim. One hundred nineteen different genotypes after being treated with suitable fungicide were sown at the appropriate depth in two replications. These cup sown seeds were subjected to four treatments and incubated at 25^{o} Celsius with white light for around 16 hours and 8 hours of darkness. Cups were filled entirely with water till the soil become saturated and a constant level of water for each treatment for predefined duration was maintained by continuous addition of water. On completion of each duration of treatment, excess moisture was removed and the seeds were allowed to germinate under optimum aerobic condition. Germination data was recorded for each of the different treatments. Different treatments employed for the pre-emergence screening of germplasm were presented in Table 3.2.

Table 3.2: Pre emergence treatments with their duration of waterlogging

S. No.	Treatment	Duration of waterlogging (days)
1	Control	0
2	Waterlog-1 (W1)	3
3	Waterlog-2 (W2)	6
4	Waterlog-3 (W3)	9

3.3 Pot experiment

One hundred nineteen inbred lines of maize along with one accession of teosinte (*Z. mays* ssp *parviglumis*) acquired from the Department of Plant Breeding and Genetics were pot sown in the month of March in three replications. These pots were placed in an artificial pit available at Rice Experimental Area, PAU. Before placing the pots, the pit was carefully sealed with black plastic sheet to avoid any seepage or percolation. One set of 120 pots were placed outside the pit and three sets; each for different duration of waterlogging; were placed inside the pit. All the pots were provided with optimum moisture, growth supplements and nutrients for uniform germination and growth. All the pots were allowed to grow optimally until the plant reaches V_2 stage, characterized by maize seedling of 2-4 inches height with two visible leaf collar and stage is generally attained at about 18-20 days of sowing. When it was observed that more than 50% of the plants in each pot attained the V_2 stage, the plants placed inside the pit were subjected to waterlogging, while the plant placed outside the pit was grown normally under optimum soil moisture. The pit was completely filled with water such that the

level remains about 4-5 cm above the pots ensuring all the plants root portion were under water while shoot parts remain exposed to air. Water level was constantly maintained by daily supplying of water. Each set of pots inside the pit was subjected to different duration of waterlogging. Different treatments employed for the seedling stage screening of germplasm were presented in Table 3.3. Layout of experiment presented in Plate 4.1.

Table 3.3: Seedling stage (V_2) treatments with their duration of waterlogging

S. No.	Treatment	Duration of waterlogging (days)
1	Control	0
2	Waterlog-1 (W1)	3
3	Waterlog-2 (W2)	6
4	Waterlog-3 (W3)	9

Waterlog-1 was subjected to waterlogging for three days and on the fourth day these pots were removed from the pit and placed outside, while other two sets were still inside the pit. Waterlog-2 was treated for six days and pots were removed leaving behind only the third set referred as Waterlog-3, which was subjected to waterlogging for continuous nine days and after which the water level was receded allowing the plants to grow normally under aerobic condition. Appropriate plant samples and data was collected from the respective treatments at respective stage.

3.4 Phenotypic evaluation

A variety of morphological traits were recorded including germination percentage, plant survival percentage, chlorophyll content, root parameters, shoot parameters, ASI, fresh weight and dry weight. Data were collected from a total of nine different intervals of waterlogging (Table 3.4) and all further analysis were performed accordingly.

Table 3.4: Different growth stages along with interval of waterlogging for data recording

S. No.	Interval of waterlogging (days)	Growth stage
1	3 days of control (T8)	3 days after plant attain V_2 stage
2	3 days of waterlogging (T9)	3 days after plant attain V_2 stage
3	6 day of control (T5)	6 days after plant attain V_2 stage
4	6 days of waterlogging (T6)	6 days after plant attain V_2 stage
5	3 days of waterlogging+3 days under optimum moisture (T7)	6 days after plant attain V_2 stage
6	9 day of control (T1)	9 days after plant attain V_2 stage
7	9 days of waterlogging (T2)	9 days after plant attain V_2 stage
8	3 days of waterlogging+6 days under optimum moisture (T4)	9 days after plant attain V_2 stage
9	6 days of waterlogging+3 days under optimum moisture (T3)	9 days after plant attain V_2 stage

3.4.1 Germination percentage

Five seeds per pot were sown and germination data was recorded counting the number of emerged seedlings up to 10 days of sowing and germination percentage was calculated as:

$$\text{Germination \%} = \frac{\text{No. of seeds germinated}}{\text{No. of seed sown}} \times 100$$

3.4.2 Plant mortality (%)

Plant mortality was accessed based on number of maize seedlings survived before waterlogging and number of plants standing in the field. It was calculated as:

$$\text{Mortality \%} = \frac{\text{Total plants before waterlogging} - \text{No. of plants standing after waterlogging}}{\text{Total plants before waterlogging}} \times 100$$

Based on the mortality data, genotypes were grouped into different groups of tolerance. Lines showing cent percent mortality after 9 days of waterlogging at seedling stage were not taken into any further experiments. From the remaining genotypes with varying level of tolerance, 54 such lines were selected that were the representative of the entire genotypes and these lines were included in all the phenotypic evaluations and further analysis.

3.4.3 Chlorophyll content (SPAD)

Chlorophyll content of the plants was measured using SPAD meter. SPAD readings were taken using Apogee MC-100 Chlorophyll Content Meter from all the four treatments and mean value obtained from three readings were taken. Readings were taken in SPAD (Minolta Special Product Analysis Division) unit. Readings were taken at 6 days after the removal of stress for Treatment 1, 3 days after the removal of stress for Treatment 2 and immediately after the removal of stress for Treatment 3. For the control set, the reading was recorded 9 days after the plants attained the V_2 stage.

3.4.4 Fresh weight (g)

Plants were uprooted immediately after each treatment and shoot and root portion were separated and fresh weight of sample was recorded in grams using standard weighing balance.

3.4.5 Dry weight (g)

After the fresh weight were noted, they were placed inside the paper envelope and dried over oven (80^{o} C) till the plants become crumbled showed no change in weight on further drying. Dry weight was measured using weighing balance in grams.

3.4.6 Root and shoot length of seedlings (cm)

The uprooted plants were cut into root and shoot fractions and individual length of each fraction was measured with the help of scale. Shoot length was measured from the point on the ground to the tip of the longest leaf and root length was measured from the collar region to the tip of the longest root. Data was recorded in cm.

3.4.7 Root trait analysis: area (square cm), volume (cubic cm)

Root trait analysis was performed by using root scanner. Root images were collected from the EPSON Expression 12000XL scanner and images were analyzed using Biovis PSM root software. To minimize the root breakage, plants sown in control plot as well as under optimum moisture were first dipped in water until the soil became saturated and gently pulled. These roots were then washed gently over the slow flowing tap water and collected in falcon tube containing 70% ethanol solution and stored until further analysis. The software performs the analysis and data for projected area (square cm), total length (cm), number of root tips, forks and segments, diameter (cm) and volume (cubic cm) were recorded.

3.4.8 Shoot area (square cm)

Shoot area of each plant was measured using the EPSON Expression 12000XL scanner and image analyzed using Biovis PSM leaf software. The software analyzes the image and data for shoot area were obtained in square cm.

3.4.9 Anthesis-Silking interval (days)

The difference in number of days between silking and tasseling was recorded from each of control, 3 days of waterlogging 6 days of waterlogging and 9 days of waterlogging treatments. ASI was recorded based on the data from days to 50% anthesis and days to 50% silking.

3.5 Selection Indices:

Selection based on the single trait analysis is the most common approach in the plant breeding. However plant system is a complex unit and each factor is the outcome of number of parameters. In the process of making selection, plant breeders have to look after a number of traits to decide its overall genetic value. Decision made by observing mere one parameter often lead to ambiguous results and thus making selection ineffective. To overcome the bottleneck, selection indexes were introduced to make the balanced selection decision by combining a number of traits with their economic weights to develop a single selection index value for each of the line considered. A number of parameters were taken care of that plays a certain role in the selection system. In the current study to select maize inbred lines against waterlogging, a number of parameters were considered. The analysis was performed in

RIndSel v 3.0 (Pacheco *et al* 2017) software and Base Linear Phenotypic Selection Index (BLPSI) and Eigen Selection Index Method (ESIM) were calculated using phenotypic information only. The economic weight for each trait considered for BLPSI was assigned to be 1 and with selection intensity of 30% each calculation was carried out. A single value was generated for each of the line comprising all the phenotypic information and higher values were considered for the selection.

Both of the selected hypothesis are based on phenotypic information only and provide us with the information to predict net genetic merit to maximize the selection response. The only difference between these theories is ESIM do not require the economic weight for each trait to be known, while the same is the important parameter to be fed for BLPSI.

For BLPSI index,

$$I = wy,$$

where w is a known vector of economic values and y is a vector of phenotypic values.

For ESIM index,

$$I = b' y',$$

where b' is the unknown index vector of coefficients and y' is known vector of trait phenotypic values.

BLSPI is the simplest linear phenotypic selection index (LPSI) developed by Williams in 1962 and has been originally given by Smith (1936), Hazel and Lush (1942), and Hazel (1943). ESIM on the other hand has been developed by Cerón-Rojas *et al* (2008).

3.6 Multi-trait stability index (MTSI)

MTSI is computed to evaluate the lines for stability and performance across several traits considered. WAASB (Weighted Average of Absolute Scores of BLUPs) index proposed by Olivoto *et al* (2019).

$$\text{WAASB} = \frac{(rG_i \times \theta_Y) + rW_i \times \theta_S)}{\theta_Y + \theta_S}$$

where WAASB is the selection index for genotype that ranks the genotypes considering performance and stability; rG_i is rescaled values for dependent variable and rW_i for *WAASB*; θ_S and θ_y are the weights for *WAASB* and dependent variable, respectively. R package 'metan' was used to measure the index and graphically represent the output.

3.7 Waterlogging tolerance coefficient (WTC):

WTC is an important parameter for screening genotypes against waterlogging stress (Liu *et al* 2010). Larger WTC value represents higher tolerance, while lower value shows the susceptible nature of the genotypes. WTC is calculated as follows:

$$\text{Waterlogging tolerance coefficient (WTC)} = \frac{\text{Mean value of parameters measured in waterlogged seedlings}}{\text{Mean value of parameters measured in control seedlings}}$$

3.8 DUS (Distinctness, Uniformity and Stability) character data collection

3.8.1 Plant materials

Out of 119 inbred lines involved in our experiments, a subset of eighty different genotypes belonging to different heterotic pools were sown in the experiment area of Plant Breeding and Genetics. All recommended package of practices was followed. The experimental materials procured from the Department of Plant Breeding and Genetics for DUS testing are enlisted in Table 3.5 below:

Table 3.5: List of genotypes under DUS evaluation

CML 32	EML 285	I 182	LM 14	PML 503	SE 549
CM 139	l 105A	I 183	LM 16	PML 892	SE 557
CM 140	l 108	I 187	LM16 Zp-1	PML 920	SE 563
CML 451	l 121	I 188	LM 20	PML 1012	SE 567
EL 1	I 132	I 190	LM 21	PML 1224	SE 571
EL 2	I 161A	I 200	LM 22	PML 1225	SE 601
EML 57	I 164	I 202	LM 23	PML 1229	SE 603
EML 122	I 165	I 205	LM 24	PML 1230	SE 604
EML 126	I 169	I 206	PML 41	PML 1231	SE 607
EML 132	I 171	I 230	PML 243	SE 503	SE 610
EML 240	I 172	I 231	PML 333	SE 533A	SE 613
EML 272	I 173	LM 6	PML 368	SE 544	SE 616
EML 282	I 177	LM 13	PML 387	SE 547	SE 617
EML 283	I 179				

3.8.2 Experimental layout

The experiment was conducted in Randomized Block Design (RBD) with three replications with row length of 4m. The plant to plant spacing was 20cm and row to row spacing was 60cm. Observations for a genotype were made from 18 plants (6 plants from each replication) following the guidelines as per PPV & FR authority (Anonymous 2007).

3.8.3 Characteristics used for observation

Observations were recorded at different growth stages and taken from the different plant parts. Four types of assessments were followed;

1. VS: Visual assessment by observations of individual plants or parts of plants

2. VG: Visual assessment by a single observation of a group of plants or parts of plants
3. MS: Measurement of a number of individual plants or parts of plants
4. MG: Measurement by a single observation of a group of plants or parts of plants

For visually assessed characteristics, no statistical methods were employed and their expression level was used for assessment of their distinctness.

A total of 28 morphological characteristics were taken into account to distinguish 80 maize inbred lines which included 20 visually assessed characteristics and 8 measurable characteristics. Twenty visually assessed and eight measurable DUS traits were recorded in field condition at their respective crop growth period. Traits like Plant height, Width of leaf blade, anthesis period, length of tassel, silking period, cob length, cob diameter and grain weight were 8 measurable DUS characters. Vernier Calliper was used for taking cob length and cob diameter, while a long (1 meter) scale was used for plant height and leaf blade width data recording. 1000 grain weight was measured using standard weighing balance.

3.8.4 Analysis of Variance (ANOVA) for measurable DUS characteristics

The lines subjected to DUS characterization were sown in three replications in Randomized Block Design (RBD). All the measurable characteristics were recorded from six plants for each line and from each replication. An average reading was calculated and subjected to one factor analysis. Significant differences were observed among the inbred lines for all the measurable characters. ANOVA for eight measurable DUS characters was calculated using R software package.

3.8.5 Descriptive Statistics for measurable DUS characters

Based on the mean data calculated for the measurable traits, maximum, minimum, critical difference, standard error and coefficient of variation were calculated. This analysis was performed in OPSTAT, an online statistical analysis tool (Sheoran *et al* 1998).

3.8.6 Clustering and Grouping of the maize inbred lines

Twenty different visually assessed DUS descriptors and eight different measurable DUS descriptors were employed to generate a Euclidian dissimilarity coefficient matrix followed by clustering using ward.D2 algorithm with the aid of R Studio software package.

3.8.7 Correlation between several measurable DUS descriptors

Eight measurable DUS descriptors were subjected to correlation study based on mean values and thus obtained correlation coefficient between the traits were plotted using RStudio.

List of DUS characteristics along with their expression and stage of observation is presented in APPENDIX-I.

3.9 ROL (Radial Oxygen Loss) barrier formation

A visual test was conducted to determine the ROL barrier formation ability of the genotypes under waterlogging stress (Armstrong and Armstrong 1988). Agar 0.1% (w/v) solution was prepared in distilled water and boiled in microwave till the solution turned transparent. The solution was taken out and allowed to cool down. To this solution methylene blue was added @ of 13 g per litre of solution. The solution turned deep blue in colour. To reduce the dye to colourless, 130 mg of 1-1 sodium dithionite ($Na_2S_2O_4$) was added which turned the solution transparent. This transparent solution was placed in 100 ml falcon tubes. Now genotypes subjected to waterlogging were uprooted, roots were trimmed off leaving behind a single root and the plant was dipped inside the falcon tube containing reduced solution. The plants were placed in such a way that the shoot portion was exposed to air and the root-shoot junction was submerged about 50 mm below the surface of solution. The plants were kept for around 45 minutes and were observed for staining. The root portion leaching the oxygen turned into blue as the methylene blue gets oxidized and root got stained, while the roots having barrier against such ROL remained colourless.

3.10 Observation of cross-sections for aerenchyma

Genotypes showing extreme level of tolerance and susceptibility against waterlogging along with *Z. mays spp parviglumis* were selected and uprooted. These plants were placed in beaker containing water. Adventitious root was selected and different cross sections were made along three different segments of the root. One section from basal region (with in 15-20 mm below root-shoot junction), another section from the intermediate region and third section was made from the apex region (within 10-15 mm behind the root tip). Cross sections were made with the help of razor blade and stained with acetocarmine. These sections were then viewed under microscope at 10X magnification and image was captured with a CCD camera linked to a computer. The percentage aerenchyma formation was determined using ImageJ software (Schneider *et al* 2012).

3.11 Orthologous sequence identification

The sequence information of *Sub1A* and *Snorkel* gene of rice was collected from the literature (Xu *et al* 2006, Hattori *et al* 2009) and corresponding orthologous sequences in the maize was searched by using BLAST tool of NCBI database (Altschul *et al* 1990). The coding sequence (CDS) were retrieved from each of the search results and these sequences were used to design new gene specific markers for qRT-PCR experiment.

3.12 Primer designing

Our target genes identified from NCBI BLAST were used for designing gene specific primers for qRT-PCR experiment. Primer3 (Untergasser *et al* 2012) online primer designing

tool was employed for designing of the primers. Care was taken such that the annealing melting temperature of forward and reverse primer of each marker are at par and problem of hairpin loop formation or primer dimer formation was eradicated by selection of such primers only which follow our requirements. List of primers designed and their sequences are shown in Table 3.6.

Table 3.6: Primers used for gene expression study using qRT-PCR

Gene name	Forward primer	Reverse primer
Sub1A CDS 1 Maize ortholog	GAAGGCATTGATGACGACCT	CCCTCAACCATGACTCCACT
Sub1A CDS 3 Maize ortholog	GACCTCGAGGAACAAGAGCA	AAGAGGAGCGAGTCGAACAG
Housekeeping / control gene (18s)	AGTTTGAGGCAATAACAGGTCT	GATGAAATTTCCCAAGATTACC

3.12.1 Primer dilution

The primers were custom synthesized from SahaGene (India). The primer was dissolved in appropriate amount of nuclease free water to obtain a main stock of 100 µM following the manufacturer's instructions. For working solution, primers were diluted to 5 µM by addition of required quantity of nuclease free water.

3.13 Genotyping using SSR markers

3.13.1 Collection of samples

Out of 119 inbred lines screened for waterlogging, ninety-five different genotypes along with one *Z. mays spp parviglumis* showing varying level of tolerance against waterlogging were selected for DNA extraction. Young leaves from the selected lines from the control set were used for sample collection. The leaves to be collected were carefully cleaned with tissue paper to remove any dirt adhered and avoiding any damaged or diseased portion, leaves were cut with sterilized scissor and wrapped in aluminum foil with proper labelling. The samples were immediately placed in ice box and stored at -80^{o}C until taken out for crushing.

3.13.2 DNA extraction:

The collected leaf samples were used for total genomic DNA extraction using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Sagai-Maroof *et al* 1984). Samples stored at -80^{o} C were taken in clean sterilized mortar and crushed using liquid nitrogen with the help of sterilized pestle. The leaf samples were ground into fine powder and transferred into labelled 2 ml centrifuge tube. Thus, transferred powder was now homogenized with 800 µl of CTAB preheated at 65^{o} C containing 2% β-Mercaptoethanol. These tubes were incubated at 65^{o} C for about an hour with intermittent shaking in each 15 minutes. After the samples were removed

from the water-bath, 800 µl of Chloroform: Iso-amyl alcohol solution made in a ratio of 24:1was added to each tube and these tubes were placed in shaker for next 40-45 minutes. Now these tubes were centrifuged at 10000 rpm for 15 minutes resulting in the separation of aqueous and non-aqueous layer inside the centrifuge tube. The upper aqueous layer was carefully pipetted out avoiding any of the debris and transferred into another 1.5 ml sterilized centrifuge tube and the 2 ml tubes with debris settled at the bottom were discarded. To this supernatant collected in 1.5 ml tube, equal volume of chilled isopropanol was added and the tube was gently inverted for uniform mixing up of the two liquids. The samples were then stored at -20^{o} C for at least half an hour. Whitish thread like precipitation was visible in each tube and these tubes were centrifuged at 10000 rpm for 15 minutes. Pellet of precipitated DNA gets settled at the bottom and a clear aqueous liquid at the top was seen. The supernatant was discarded and the DNA pellets were subjected to washing with 70% ethanol to remove any salt and impurities adhered to the pellet. Two washings of ethanol were done by adding 300µl of 70% ethanol and centrifuged at 10000 rpm for 10 and 5 minutes in first and second washing, respectively. Ethanol was discarded after each washing and tubes containing pellet of DNA were left with its lid open to allow evaporation of excess ethanol and drying of pellet. After 4-5 hours of drying at room temperature, 100 µl of TE buffer was used for dissolving the DNA pellet and left overnight in room temperature for proper dissolution. On the next day to remove the RNA contamination from the DNA, RNase (10 mg/ml), approximately 10 µl, in each tube was added and the tubes were incubated for an hour at 37^{o} C. This contamination free DNA was stored at -20^{o} C for all further study and longer storage. Composition of CTAB and TE buffer is presented in Table 3.7.

Table 3.7: Composition of CTAB and TE Buffer

Components	**Working concentration**	**Amount required for 1 Litre**
CTAB composition		
CTAB (Cetyl Trimethyl Ammonium Bromide)	2%	20 g
EDTA, pH 8.0	20 Mm	7.44 g
NaCl	1.4 M	81.9 g
Tris base	100 Mm	12.114 g
β-Mercaptoethanol (To be added just before use)	0.2%	
TE composition		
Components	Working concentration	Amount required for 200 ml
Tris base (pH 8.0)	1 M	0.316 g
EDTA	0.5 M	0.074 g

3.13.3 Quantification of genomic DNA

Extracted DNA were quantified using agarose gel electrophoresis unit and nanodrop.

3.13.3.1 Quantification using gel electrophoresis

For gel electrophoresis, 0.8% agarose gel was prepared by dissolving 0.8 g of agarose in 100 ml of 0.5X TBE buffer. TBE buffer initially available at 10X was diluted to 0.5X by addition of distilled water. The mixture was heated in microwave so that all the agarose got dissolved and a clear liquid was obtained. This solution was taken out and cooled with continuous stirring. When the solution became bearable to touch, ethidium bromide (5 µl/100ml) was added and stirred for uniform mixing up. Now the gel was poured in cast tray containing the comb and sealed with tape and allowed to solidify for about 40-45 minutes. After the gel got solidified, the sealing tape was removed and the gel along with cast tray was put inside the electrophoresis unit containing the same 0.5X TBE buffer such that the gel got completely submerged inside the TBE buffer and combs were removed revealing the small wells for loading of the DNA samples. For loading the DNA was added with the loading dye (1X). The bromophenol blue (loading dye comprises 0.25 per cent w/v bromophenol blue, 0.4 per cent w/v sucrose or 30 per cent glycerol in sterile water) was initially available in 6X and was diluted to 1X by addition of appropriate amount of nuclease free water. Now about 2 µl of genomic DNA along with 8 µl of 1X loading dye were thoroughly mixed and this 10µl of final DNA mixture is carefully loaded in a well using micropipette. Different DNA samples were loaded in different wells and DNA of known concentration was also loaded to compare the size and quality of the extracted DNA. Now the electrophoresis unit was run for about 4-5 hours at a constant voltage of 5 V/cm. After running for required duration, the gel was taken out from the unit and visualized using UV transilluminator and photographed using photo gel documentation system. Now the quality and size of the extracted DNA was analyzed using the band position and sharpness of DNA of known concentration. Composition of TBE buffer is presented in Table 3.8.

Table 3.8: TBE Buffer composition

Components	Working concentration	Amount required for 1 Litre
Tris Base	45 mM	109.02 g
Boric acid	45 mM	55.6 g
EDTA (pH 8.0)		7.44 g
Distilled water		Amount required to make final volume of 1 Litre.

3.13.3.2 Quantification using nanodrop

For quantification, Nanodrop-8000 system available at School of Agricultural Biotechnology, PAU was used. First the sensors were carefully cleaned using the Kimwipes

tissue and system was initialized and nucleic acid was chosen. System was initialized by loading 2 µl of nuclease free water over each sensor and calibrated using TE buffer. After calibrating the system, the sensors were cleaned carefully and 2 µl of DNA was loaded in each of the 8 sensors and data obtained on the screen were recorded. The sensors were cleaned each time a new sample was loaded. The concentration of the DNA in the sample was obtained in ng/µl and quality was judged based on 260/280 value. Value close to 1.8 was considered to be of good quality.

3.13.4 Normalization of genomic DNA

Based on the results obtained from both the gel image and nanodrop result, concentration of DNA was normalized to 20 ng/µl for further SSR analysis.

3.13.5 SSR analysis

3.13.5.1 Selection of markers

SSR markers reported to be polymorphic for waterlogging trait were selected from the previous studies (Kaur 2018). These markers were found to be associated with different traits conferring tolerance and 20 such markers belonging to different chromosomes were genotyped in diverse set of inbred lines and screened for polymorphism. List of markers and their chromosomal position is sown in Table 3.9 below:

Table 3.9: List of SSR markers employed for genotyping

S. No.	Primers	Chromosomal location	Bin	Annealing temperature (^{o}C)
1	bnlg244	9	9.02	54
2	umc1236	8	8.03	54
3	umc1782	7	7.04	53
4	umc1676	1	1.05	54
5	umc2340	9	9.03	57
6	umc2134	9	9.05	52
7	umc1033	9	9.03	52
8	umc2199	8	8.05	51
9	bnlg1812	8	8.05	51
10	umc1383	1	1.08	53
11	bnlg1007	1	1.02	52
12	umc2204	1	1.02	55
13	bnlg182	1	1.03	52
14	umc2227	1	1.04	57

S. No.	Primers	Chromosomal location	Bin	Annealing temperature (^{o}C)
15	phi001	1	1.03	60
16	bnlg1016	1	1.04	52
17	umc1021	1	1.03	54
18	umc2210	8	8.05	53
19	umc2048	3	3.10	53
20	umc1052	3	3.09	54

3.13.5.2 PCR amplification and Gel electrophoresis

In vitro PCR (Polymerase Chain Reaction) was employed for amplification of specific DNA fragment. It was carried out in 96 well Applied Biosystems™ thermocyclers. PCR reaction mixture of 15 µl containing 3 µl of template DNA (60 ng), 5 µl (2X premix) master mix, 4 µl nuclease free water and 1.5 µl of each forward and reverse primer was used for PCR amplification. Thermal profile for PCR amplification:

Initial denaturation at 94^{o} C for 4 minutes followed by 40 cycles of denaturation at 94^{o} C for 30 seconds, annealing at 51-60^{o} C (depending on the primer) for 45 seconds and extension at 72^{o} C. Final extension step at 72^{o} C for 7 minutes for complete amplification of the desired specific fragment. The product was finally hold at 4^{o} C for infinity until the sample was taken out for further storage or gel analysis. Quantity and concentration of PCR components is presented in Table 3.10.

Table 3.10: Quantity and concentration of different PCR components

Components	Stock concentration	Quantity	Final concentration
Template DNA	20 ng/µl	3 µl	60 ng
Master mix (2X premix)	2X	5 µl	-
Forward primer	5 µM	1.5 µl	0.5 µM
Reverse primer	5 µM	1.5 µl	0.5 µM
Sterile water	-	4 µl	-
Total		15 µl	-

3.13.5.3 Visualization of amplified products

The amplified PCR product was then loaded in 2.5% agarose gel prepared in 0.5% TBE buffer added with ethidium bromide (5 µl/100ml) and run at constant voltage of 5 V/cm for about 4-5 hours and the gels were visualized under transilluminator and photographed under gel documentation system. DNA ladder (DNA fragments of known size) of 100 bp was

loaded along with the amplified products for determining the approximate size of different products.

3.13.5.4 Scoring of SSR markers

All the bands visualized in the gel were critically scored and all the allelic variation present in the genome were accurately noted down. SSR marker was scored in co-dominant manner and presence or absence of specific allele was denoted as "1" and "0".

3.14 Expression analysis

3.14.1 Sample collection

Since RNA is stage and tissue specific, root tissues were targeted for our further studies. Plants at V_2 stage were subjected to 3, 6 and 9 days of continuous waterlogging and sampling was done after each treatment and corresponding unstressed plants taken as control were also collected. For sample collection, the root was immediately harvested after the plant was taken out of the water and dipped into bucket full of water to wash out the dirt and mud adhered to the root tissues and immediately dipped in 0.1% Diethyl pyrocarbonate (DEPC) water to remove any RNase activity. Now using a sterilized scissor, the root tissues were collected in sterilized labelled aluminum foil, properly wrapped and snap froze with liquid nitrogen and stored at -80^o C until they were crushed for RNA isolation. The samples were collected in duplication.

3.14.2 Isolation of total RNA

For RNA isolation all the glassware and equipment to be used were cleaned properly and dipped in 0.1% DEPC water. These treated materials were dried in hot air oven over night, wrapped in aluminum foil and autoclaved at 121^o C for 15 minutes. Mortar and Pestle to be used for crushing were stored in -80^o C until they were used for crushing. Samples kept at -80^o C were crushed in chilled mortar pestle with the help of liquid nitrogen. When the tissues were ground into fine powder, Trizol (1 ml/100g) was added to it and homogenized. The homogenized solution was allowed to liquefy by continuous stirring till the solution became thin turbid liquid. This solution was transferred into 2 ml micro centrifuge tube and 200 µl of chloroform was added per ml of trizol and mixed by vertexing the tube for couple of seconds. The solution was then incubated in room temperature for about 10 minutes. The tubes were then centrifuged at 12000 rpm for 5 minutes at 4^o C. The solution separates into top aqueous and bottom non-aqueous layer. The aqueous layer was pipetted out and transferred into 1.5 ml micro centrifuge tube. To this supernatant, chilled isopropanol in equal volume to supernatant was added and placed in ice for half an hour to allow precipitation. After the incubation in ice, the tubes were thoroughly mixed and centrifuged for 10 minutes at 12000 rpm temperature maintained at 4^o C. RNA pellets were formed at the bottom of the tube. The upper aqueous liquid was discarded and the RNA pellet was washed with 70%

DEPC-ethanol. Two washings were given with the 1ml DEPC-ethanol by centrifuging at 12000 rpm for 5 minutes at 4^{o} C in each washing. Thus, obtained clean RNA pellet was allowed to air-dry at room temperature by keeping tube's lid open and left to dry out for 2-3 hours. After the pellet was dried, DEPC-water preheated at 65^{o} C was added to dissolve the RNA. Amount of DEPC-water to be added was decided based on the pellet size and approximately 20-30 µl was added in each tube and stored at -80^{o} C for long term storage and until used for preparation of cDNA.

3.14.3 RNA quantification

RNA was quantified over both nanodrop and gel electrophoresis unit. In nanodrop, 2 µl of RNA from original stock was loaded in nanodrop using DEPC water as blank and results were recorded. The samples with 260/280 values ranging between 1.8-2.0 were considered to be of good quality and were proceeded forward for further experiments.

3.14.3.1 Denaturing formaldehyde agarose gel electrophoresis

1.5% formaldehyde agarose (FA) gel was prepared by adding 1.5 g of agarose in 100ml of 1X 3-(N-Morpholino) propane sulfonic acid (MOPS) buffer. MOPS originally available at 10X and was made to 1X by adding appropriate amount of DEPC-water. The solution was boiled in microwave until it turned into clear transparent liquid. The solution was taken out and allowed to cool down. When the temperature fell to around $50\text{-}60^{o}$ C, 1 ml formaldehyde per 100 ml of solution was added and ethidium bromide (1.5 µl) was added and allowed to cool down by continuous swirling. When the solution has cooled down, it was poured in casting tray fitted with combs and end sealed with tape. The gel was allowed to solidify, tape and combs were removed and put in electrophoresis unit containing 1X MOPS buffer. The loading solution was prepared by adding 2 µl of RNA from original stock, 2 µl of 6X loading dye and 6 µl of DEPC water. The system was run at 5 V/cm for 4-5 hours and the gel was visualized under transilluminator and photographed under gel documentation system. Composition of MOPS buffer is presented in Table 3.11.

Table 3.11: Composition of MOPS buffer

MOPS (10X) preparation		
Components	**Concentration**	**1 Litre**
MOPS (mol. wt.= 209.26 g/mol)	0.2 M	41.86 g
Sodium Acetate (mol. wt.=82.03 g/mol)	0.05 M	4.1 g
Na_2EDTA (mol. wt.=372.24 g/mol)	0.01 M	3.72 g
Distilled water	-	Added to make final volume to 1 Litre

Maintain pH=7 and autoclave the buffer.

3.14.4 cDNA preparation

cDNA was prepared by using Takara 1st strand cDNA synthesis kit. Both RNA and cDNA synthesis kit were thawed in ice. In a micro PCR tube, 1 µl of oligodT primer, 1 µl of dNTP mixture, 2 µl of RNA from original stock and 6 µl of RNase free water was added to make a final volume of 10 µl. This tube was then incubated at 65° C for 5 minutes and chilled on ice for 10 minutes. Now to this 10 µl solution, 4 µl of 5X prime script buffer, 0.5 µl of RNase inhibitor and 1 µl of Prime Script RT was added. Finally, 4.5 µl of RNase free water was added to make a final volume of 20 µl. This final volume was mixed gently and incubated in thermocycler at 30° C for 10 minutes followed by 52° C for 60 minutes and finally at 70° C for 15 minutes. Thus, obtained cDNA was diluted to final concentration of 100 ng/µl and used for qRT PCR.

3.14.5 Quantitative Real Time (qRT) PCR reaction

qRT PCR was performed in StepOnePlus Real-Time PCR system by Applies Biosystems. The reaction was conducted using TB Green Premix Ex Taq by Takara. Reaction mix of 12 µl was prepared by adding 4 µl of diluted cDNA, 1.5 µl of forward primer, 1.5 µl of reverse primer and 5 µl of TB Green Premix. Primers specific Actin gene (J01238.1) and 18s (XR_004851176.1) of maize were used as internal control in qRT-PCR experiment. Three technical replicates of each sample were used and following thermal profile was followed:

Initial denaturation at 95° C for 30 seconds followed by 40 cycles of amplification (denaturation at 95° C, primer annealing at 60° C for 1 minutes and primer extension at 72° C for 40 seconds). For the melt curve, the thermal profile was 95° C for 15 seconds, 60° C for 1 minutes and 95° C for another 15 seconds. Components used in qRT-PCR is presented in Table 3.12.

Table 3.12: Components used in qRT-PCR

Components	Stock concentration	Quantity	Final concentration
Template cDNA	100 ng/µl	2 µl	200 ng
TB Green Premix Ex Taq II	2X	5 µl	1X
Forward primer	10 µM	1.2 µl	1µM
Reverse primer	10 µM	1.2 µl	1µM
Sterile water	-	2 µl	-
Total		12 µl	-

The data obtained from the qRT PCR was normalized using the value of internal gene control and fold change was calculated by $2^{-\Delta\Delta}$ method.

3.15 Population structure analysis:

Population structure analysis was performed using STRUCTURE 2.3.4 software package (Pritchard *et al* 2000) employing admixture model-based clustering method. The parameter for running length was set as length of burn-in period as 10,000 and number of Markov Chain Monte Carlo (MCMC) reps after burn-in as 10,000. Different combinations of k value (number of clusters) with different alterations were checked and a final model was created by setting number of clusters (k = 10) with 10 different alterations for each K. To find the best K among all the obtained values, the whole data was compressed in a ".zip" file and was uploaded into the STRUCTURE HARVESTER database utilizing the Evanno method (Evanno *et al* 2005).

3.16 Association mapping for various phenotypic traits

Marker trait association was carried out using TASSEL v5 and an R package called rMVP (memory-efficient, visualization-enhanced and parallel-accelerated tool) version 1.0.6 available at http://www.r-oroject.org/. Genotyping data was converted into HapMap format and phenotypic data was stored in tab delimited format for the package to accept the data. The phenotypic data collected from different environments were merged to create a single BLUE (best linear unbiased estimates) data and used for all further analysis. This package performs the parallel-accelerated association tests of markers with phenotypes using three methods, namely general linear model (GLM), mixed linear model (MLM) and fixed and random model circulating probability unification (FarmCPU) model. Results yielded from the above analysis were presented in output table and graphically represented in Manhattan plots by scoring the $-\log_{10}(P)$ values against the genetic position of the loci and presented as quantile-quantile (Q-Q) plot. A permutation threshold of 100 was applied to the analysis.

CHAPTER IV

RESULTS AND DISCUSION

Among the several abiotic stresses, waterlogged condition leading to excessive soil moisture (ESM) is a major constraint of maize production. The plant undergoes several modifications in physiological, morphological, biochemical, anatomical and molecular levels to withstand against such stressful condition. Identification of such candidate genes associated with these alterations will contribute to maize breeding programmes in development of more stress-tolerant lines. The results from the present investigation entitled "Identification of maize genotypes tolerant to waterlogging and their characterization using rice orthologs" are presented in this chapter. Detailed discussion on selection of lines against waterlogging, genetic diversity as well as expression analysis for the rice orthologs and maker trait association for different phenotypic characters have been made using diverse set of inbred lines.

4.1 Pre-emergence screening of genotypes for waterlogging stress tolerance

Seeds of almost all the genotypes under controlled conditions showed germination above 80%. There was similar trend for germination after three days of waterlogging. But when the waterlogging stress was extended for six days, germination percentage started declining and lines showed varying degree of germination. Zaidi *et al* (2012) reported 72 hours of continuous waterlogging at pre-emergence stage to be the threshold limit for selection of line, but results from the current study showed a different trend. At 72 hours no significant variation in percentage germination was observed when compared to control. Difference in percentage germination only became evident when the treatment was extended up to 6 days. On further extending the duration of waterlogging, no genotypic variation was observed as all the lines showed susceptible response. None of the seeds, except for I 172, showed germination after 9 days of continuous waterlogging. List of genotypes used for pre-emergence screening along with their percentage germination at different duration of waterlogging are presented in Table 4.1. The experiment was carried out in two replications along with control. All the genotypes, except *Z. mays spp parviglumis*, were screened for pre-emergence waterlogging.

Table 4.1: Genotypes with their germination percentage at different duration of waterlogging

Genotypes	Germination percentage				Genotypes	Germination percentage			
	Control	T1	T2	T3		Control	T1	T2	T3
EML 272	50	30	0	0	I 105A	80	80	40	0
I 231	80	50	0	0	I 173	50	40	40	0
CM 139	60	50	0	0	PML 1224	90	70	40	0
EL 1	50	40	0	0	PML 1225	90	60	40	0
EL 2	60	40	0	0	PML 243	90	60	40	0
I 164	40	30	0	0	PML 82	80	40	40	0
I 165	60	50	0	0	EML 124	80	80	50	0
I 182	50	20	0	0	EML 57	90	90	50	0
I 188	60	50	0	0	AVL13119	90	80	50	0
PML 1231	50	40	0	0	I 162	90	90	50	0
SE 563	30	0	0	0	I 185	90	80	50	0
SE 571	50	30	0	0	LM 12	100	100	50	0
EML 285	80	30	10	0	LM 23	100	80	50	0
I 230	80	80	10	0	PML 373	90	70	50	0
EML 283	80	20	10	0	PML 400	90	80	50	0
SE 604	80	80	10	0	VL 1010764	90	90	50	0
SE 607	90	80	10	0	EML 125	100	70	60	0
I 179	50	40	10	0	EML 52	100	90	60	0
I 161A	100	70	10	0	AVL13056	90	90	60	0
LM 14	50	40	10	0	I 168	90	80	60	0
LM 24	50	40	10	0	I 170	90	80	60	0
PML 503	90	90	10	0	I 192	90	90	60	0
SE 547	80	70	10	0	JCY 20	90	90	60	0
SE 617	80	60	10	0	LM 13	90	70	60	0
CHT 219301	90	70	20	0	LM 26	80	80	60	0
NL 219608	90	60	20	0	PML 1229	90	90	60	0
I 202	60	50	20	0	PML 333	80	80	60	0
EML 130	60	40	20	0	PML 28	100	100	60	0
LM 16 Zp-1	50	50	20	0	PML 368	100	70	60	0
EML 279	40	40	20	0	SE 503	80	80	60	0
EML 126	50	40	20	0	EML 240	90	80	70	0

Genotypes	Germination percentage				Genotypes	Germination percentage			
	Control	T1	T2	T3		Control	T1	T2	T3
SE 544	60	50	20	0	AVM11207	100	90	70	0
I 183	90	80	20	0	I 169	90	70	70	0
CML 32	50	50	20	0	I 172	100	100	70	10
CML 451	90	80	20	0	I 189	100	80	70	0
I 121	80	80	20	0	I 200	100	100	70	0
I 132	90	50	20	0	I 206	90	80	70	0
I 177	90	50	20	0	LM 16	100	100	70	0
SE 610	80	70	20	0	LM 22	90	70	70	0
LM 11	100	90	20	0	PML 1012	80	80	70	0
PB Sathi	90	70	20	0	PML 1228	90	90	70	0
PML 1230	100	30	20	0	PML 224	90	80	70	0
PML 41	90	60	20	0	PML 387	100	100	70	0
PML 384	80	70	20	0	PML 898	100	100	70	0
SE 549	90	80	20	0	SE 616	80	70	70	0
SE 613	80	70	20	0	EML 53	100	100	80	0
NL 219605	90	50	30	0	CM 140	80	80	80	0
SE 601	80	80	30	0	I 171	90	80	80	0
EML 122	80	70	30	0	I 204	100	90	80	0
EML 128	70	50	30	0	I 205	90	90	80	0
EML 282	100	100	30	0	LM 17	90	80	80	0
EML 132	90	60	30	0	LM 20	90	80	80	0
I 190	80	70	30	0	LM 5	80	80	80	0
LM 21	90	90	30	0	LM 6	90	80	80	0
SE 603	80	60	30	0	LM 19	100	80	80	0
PML 920	90	80	30	0	PAC 753	100	80	80	0
SE 533A	50	30	30	0	PML 1241	90	90	80	0
SE 557	80	50	30	0	PML 169	100	90	80	0
SE 567	100	70	30	0	I 187	100	90	90	0
I 108	60	60	40	0					

T1: Three days of continuous waterlogging, T2: Six days of continuous waterlogging, T3: Nine days of continuous waterlogging

4.2 Screening at seedling stage for waterlogging stress tolerance

All inbreds from pre-emergence screening along with one accession of teosinte "*Z. mays ssp. parviglumis*" were sown in pots in four sets and kept inside the pit. Six seeds from

each line were sown in one pot. Seeds in all the four sets were allowed to germinate and grow under optimum moisture condition till the seedlings reached V_2 stage, that is characterized by presence of two well developed leaves with leaf sheath around. At this stage seedlings were subjected to 3, 6 and 9 days of waterlogging. After each subsequent treatment of 3, 6 and 9 days of waterlogging, each set was taken out of the pit and subsequent data was recorded. Seedlings subjected to waterlogging for different intervals showed varying degree of tolerance and varying survival percentage. Seventeen genotypes had zero survival at 9 days of waterlogging were deemed as highly susceptible and hence were not carried forward in the experiments. Other lines showed varying level of tolerance and based on their performance at different intervals of waterlogging, these were grouped together and 54 lines with different tolerance level were used in all further experiments.

4.2.1 Plant mortality

Plant mortality was calculated based on the number of plants germinated and number of plants surviving after the removal of stress. Waterlogging stress for three days at seedling stage did not show significant effect over plant survival. Almost all the plants had zero mortality, though few susceptible genotypes showed around 10-20% mortality rate. At 6 days of stress, susceptible lines started showing higher mortality rate, reaching as high as 50 percent and above. At 9 days, highly susceptible lines almost died out showing no survival at all. These lines were categorized as highly susceptible. Tolerant lines showed 100 percent survival even at 9 days of waterlogging. Lines with per cent mortality between 25 to 50% were deemed as moderately tolerant and with mortality between 50-75% were categorized as moderately susceptible. Number of genotypes falling in each of different groups based on their mortality percentage are shown in Table 4.2 and Fig. 4.1. The percentage mortality data is presented in Table 4.3. Genotypes CML 32, NL 219605, I 183, EML 128, EML 130, I 231, SE 610, LM 21, I 105A, I 108, I 177, I 188, PAC 753, PML 1224, PML 1225, SE 547 and SE 613 showed no survival after 9 days of waterlogging and were discarded for all further data collection. Classification of genotypes was carried out based on the percentage survival data under the stress condition as adopted by earlier researcher Amin *et al* (2015).

Table 4.2: Scale used for categorization of lines based on mortality percentage

S. No.	Scale (Per cent mortality after 9 days of waterlogging)	Remarks	No of lines
1	0-25	Highly Tolerant	68
2	25-50	Moderately Tolerant	18
3	50-75	Moderately Susceptible	11
4	75-100	Highly Susceptible	23
Total			120

Plate 4.1: a. Sowing of seeds in pots and seedlings allowed to grow under optimum condition till V_2 stage, b. Waterlogging stress applied at V_2 stage for 3, 6 and 9 days, c. Tolerant line 15 days after removal of waterlogging stress, d. Susceptible line 15 days after removal of stress, e. '*Z. mays spp parviglumis*' accession of teosinte showing tolerance.

A radar graph was constructed to find relation between per cent germination at 6 days of pre-emergence waterlogging and survival at 9 days of waterlogging. No significant relationship was found. Some lines having higher germination at pre-emergence still had lower survival rate after 9 days of waterlogging and vice versa. The relation is diagrammatically presented in Fig. 4.2. Some of the lines showing consistent result in both the conditions were EML 240, EML 53, I 169, I 172, I 204, LM 16, LM 6, PML 1228, PML 387 and SE 616.

4.2.2 SPAD (Soil Plant Analysis Development) value

After the removal of the subsequent waterlogging stress, SPAD data was collected using MC-100 Chlorophyll Concentration Meter from Apogee Instruments. Data collected in SPAD from four different treatments were presented in Table 4.4. The chlorophyll content as recorded by SPAD drastically reduced in response to waterlogging. The percentage reduction in the chlorophyll content was as high as 43.32% for highly susceptible line (I200) at 3 days of waterlogging, while the tolerant lines were able to maintain their chlorophyll level to high extent and percentage reduction was as low as 2.68% for EML 57. On further increasing the duration of waterlogging to 6 and 9 days, these values went on decreasing and lowest values were recorded at 9 days of waterlogging. The chlorophyll reduction was as high as 73.17% for I162 at 9 days of waterlogging and least was 12.74% for EML 285. These results were in complete arrangement with the results obtained by Ren *et al* (2016a). Chlorophyll content reduction was seen even at the early duration of waterlogging and the susceptible lines showed higher percentage reduction, while the tolerant lines were able to overcome these ill effects more effectively and maintain the chlorophyll level to some extent as shown in Fig. 4.3.

Genotypes showing severe reduction (>60%) in SPAD reading at 9 days of waterlogging were EML 279, EML 52, CHT119075, I 200, I 206, I 162, LM 12, LM 20, LM 5, PML 1229, PML 1241, PML 169 and PML 898. Some lines showed tolerance and exhibited minimal reduction in SPAD value (<20%) even at 9 days of waterlogging. These lines were EML 285, EML 124, EML 125, AVL13119, I 172, and *Z. mays spp parviglumis*.

Table 4.3: Mortality percentage after different duration of waterlogging treatment

Genotypes	Mortality percentage				Genotypes	Mortality percentage			
	Control	T1	T2	T3		Control	T1	T2	T3
EL 2	0	0	0	0	SE 616	0	0	0	0
LM 16 Zp-1	0	0	0	0	LM 17	0	0	0	0
PML 333	0	0	0	0	LM 6	0	0	0	0
CM 139	0	0	0	0	I 168	0	0	0	0

Genotypes	Mortality percentage				Genotypes	Mortality percentage			
	Control	T1	T2	T3		Control	T1	T2	T3
EML 132	0	0	0	0	SE 503	0	0	0	0
CML 451	0	0	0	0	AVL13056	0	0	0	0
EL 1	0	0	0	0	EML 285	0	0	0	0
EML 122	0	0	0	0	I 185	0	0	0	0
EML 124	0	0	0	0	AVL13119	0	0	0	0
I 202	0	0	0	0	I 204	0	0	0	0
I 230	0	0	0	0	I 179	0	0	0	20
EML 240	0	0	0	0	LM 16	0	0	20	20
EML 283	0	0	0	0	LM 24	0	0	0	20
SE 601	0	0	0	0	PML 1230	0	20	20	20
SE 603	0	0	0	0	PML 1231	0	0	25	20
SE 604	0	0	0	0	PML 28	0	0	20	20
SE 607	0	0	0	0	PML 387	0	0	0	20
EML 57	0	0	0	0	SE 567	0	20	20	20
I 121	0	0	0	0	VL 1010764	0	0	0	20
I 132	0	0	0	0	PML 368	0	0	20	20
I 161A	0	0	0	0	PML 373	0	0	0	20
I 165	0	0	0	0	PML 384	0	0	0	25
I 169	0	0	0	0	I 171	0	0	0	37.5
I 172	0	0	0	0	I 205	0	10	20	37.5
I 173	0	0	0	0	LM 20	0	12.5	20	37.5
I 190	0	0	0	0	LM 22	0	10	10	37.5
I 192	0	0	0	0	LM 5	0	10	20	37.5
EML 126	0	0	0	0	EML 52	0	0	0	40
JCY 20	0	0	0	0	LM 12	0	0	20	40
LM 11	0	0	0	0	LM 13	0	0	20	40
LM 26	0	0	0	0	PML 1012	0	10	20	40
Z. mays spp parviglumis	0	0	0	0	SE 571	0	20	20	40
PB Sathi	0	0	0	0	I 187	0	10	20	42
PML 1228	0	0	0	0	CHT 219301	0	20	20	50
PML 243	0	0	0	0	CM 140	0	40	40	50
PML 41	0	0	0	0	EML 279	0	0	20	50
PML 503	0	0	0	0	I 164	0	0	0	50
SE 533A	0	0	0	0	I 182	0	0	0	50
SE 544	0	0	0	0	PML 1229	0	10	37.5	50

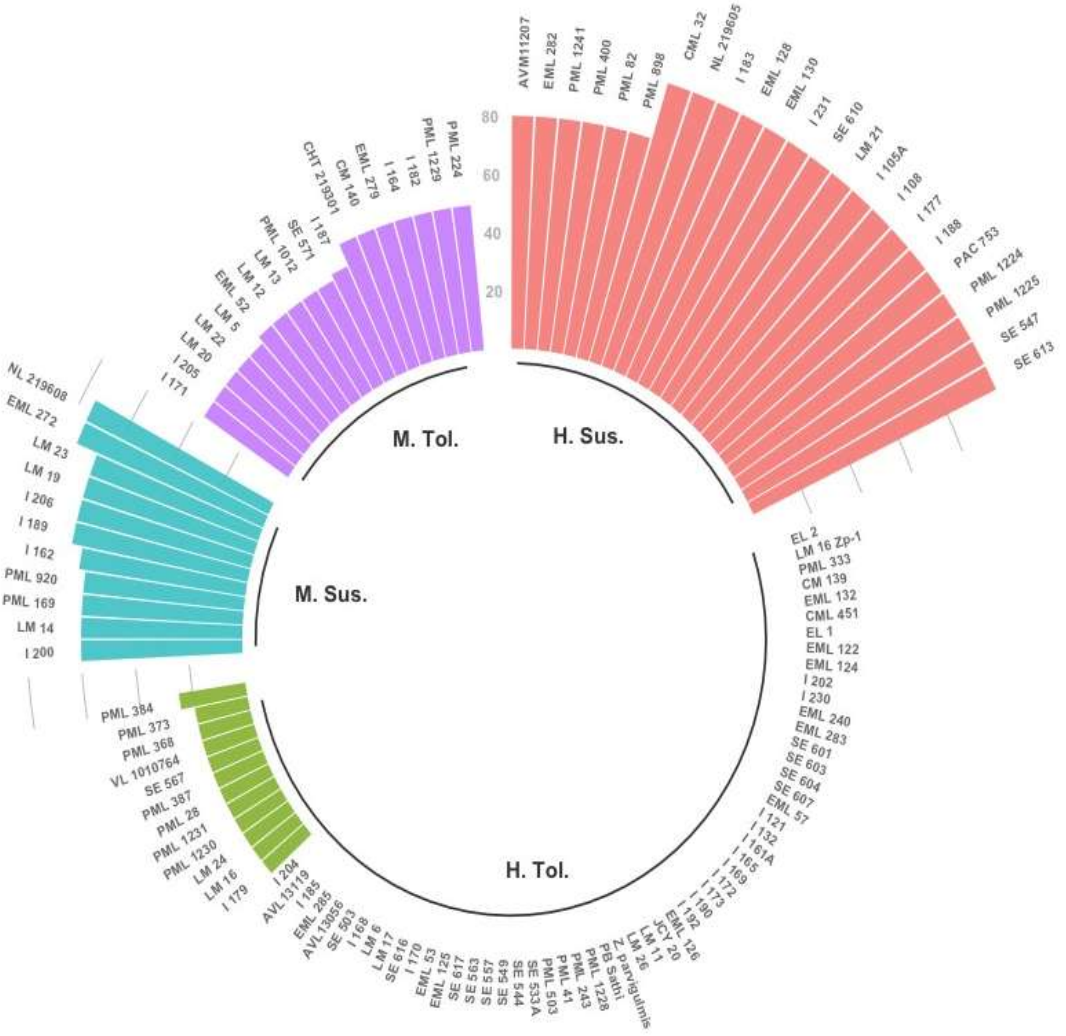
Genotypes	Mortality percentage				Genotypes	Mortality percentage			
	Control	T1	T2	T3		Control	T1	T2	T3
SE 549	0	0	0	0	PML 224	0	10	40	50
SE 557	0	0	0	0	I 200	0	20	20	60
SE 563	0	0	0	0	LM 14	0	20	20	60
SE 617	0	0	0	0	PML 169	0	0	0	60
EML 125	0	0	0	0	PML 920	0	0	20	60
EML 53	0	0	0	0	I 162	0	10	37.5	62.5
I 170	0	0	0	0	I 189	0	0	20	66.6
I 206	0	10	40	66.6	EML 128	0	0	50	100
LM 19	0	0	40	66.6	EML 130	0	0	0	100
LM 23	0	12.5	37.5	66.6	I 231	0	0	25	100
EML 272	0	50	66.6	75	SE 610	0	0	0	100
NL 219608	0	0	60	75	LM 21	0	0	0	100
AVM11207	0	10	40	80	I 105A	0	0	50	100
EML 282	0	0	0	80	I 108	0	0	33.3	100
PML 1241	0	10	66.6	80	I 177	0	0	0	100
PML 400	0	0	66.6	80	I 188	0	0	33.3	100
PML 82	0	25	75	80	PAC 753	0	0	60	100
PML 898	0	0	66.6	80	PML 1224	0	0	20	100
I 183	0	0	25	100	PML 1225	0	0	33.3	100
CML 32	0	0	40	100	SE 547	0	0	100	100
NL 219605	0	0	0	100	SE 613	0	0	0	100

T1:Three days of continuous waterlogging, T2:Six days of continuous waterlogging, T3:Nine days of continuous waterlogging

4.2.3 Seedlings morphological parameters

Sampling of the plants were done at nine different growth condition. These include 3 days control (T8), 3 days of continuous waterlogging (T9), 6 days control (T5), 6 days of continuous waterlogging (T6), 3 days of waterlogging flooded by 3 days under optimum moisture (T7), 9 days control (T1), 9 days of continuous waterlogging (T2), 3 days of waterlogging followed by 6 days under optimum moisture (T4), 6 days of waterlogging followed by 3 days of optimum moisture (T3). All the morphological parameters showed significant reduction over waterlogging. For four different intervals of waterlogging at 9 days, the highest reading was observed for 9 days of control and the lowest was recorded for 9 days of waterlogging. For three different intervals of waterlogging at 6 days, the highest value was recorded for 6 days control and lowest for 6 days of treatment, while an intermediate value was recorded for 3 days of waterlogging flooded by 3 days under optimum moisture. For 3 days of waterlogging, there was significant reduction in morphological parameters between the treatment and control. All the root and shoot morphologoical parameters considered for

the current study decline significantly over waterlogging. It is in complete agreement with the study reported by Ren *et al* (2016b) and Zaidi *et al* (2003). They presented that under waterlogging, all the morphological parameters showed decline but the susceptible lines had sharp fall in the value as compared to tolerant lines. Kaur *et al* (2019) in their study also showed that, based on the percentage decline in morphological parameters one can judge tolerant or susceptible nature of the genotypes. Waterlogging severely affects the efficiency of soil nutritients and water absoption by roots, thereby inhibiting normal growth and development. Reduction in plant height and ear height under the response of waterlogging stress had been previously established by number of researchers (Pal and Varande 1984, Chu *et al* 1995, Tripathi *et al* 2003).



H. Tol.: Tolerant, M. Tol: Moderately Tolerant, M. Sus: Moderately susceptible, H. Sus: highly susceptible

Fig. 4.1: Circular bar graph representing mortality percentage at different duration of waterlogging

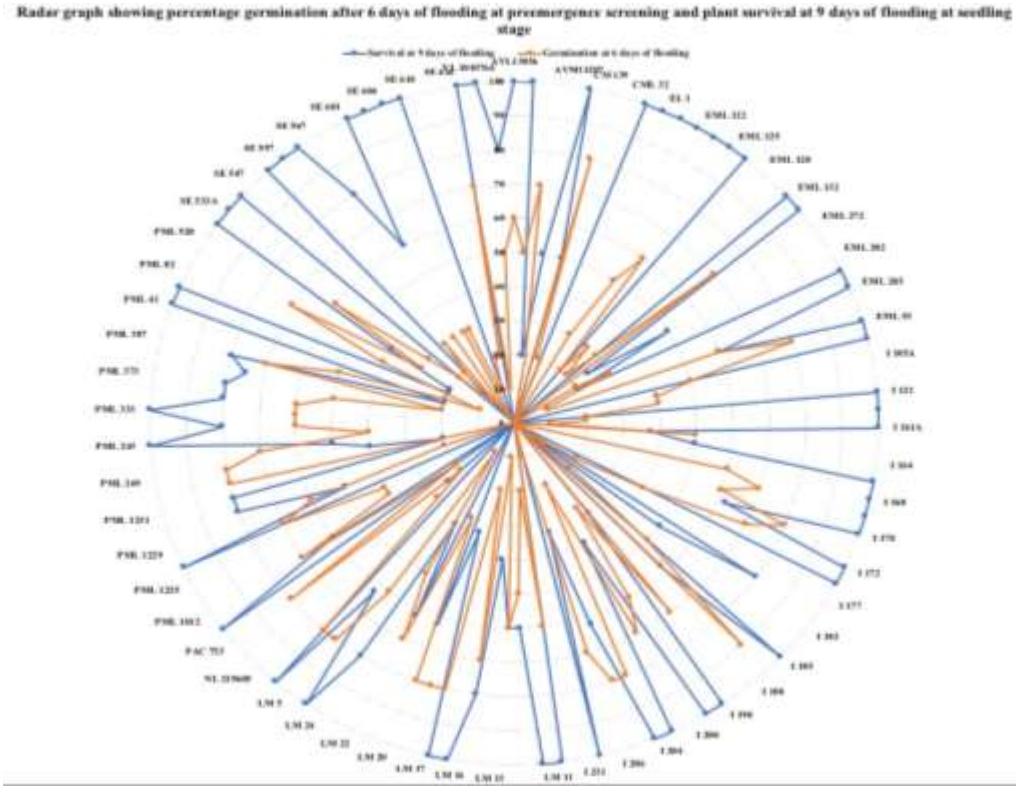


Fig. 4.2: Radar graph showing percentage survival at 9 days of waterlogging along with percentage germination at 6 days of pre-emergence waterlogging.

Table 4.4: SPAD reading of lines at various duration of waterlogging

Genotypes	At control	At 3 days water logging	% reduction	At 6 days waterlogging	% reduction	At 9 days water logging	% reduction
EML 285	32.35	30.65	5.26	29.25	9.58	28.23	12.74
EML 124	35.15	30.95	11.95	30.17	14.17	28.75	18.21
EML 125	34.55	32.52	5.88	30.25	12.45	29.21	15.46
EML 279	39.35	35.15	10.67	29.09	26.07	13.21	66.43
EML 52	39.19	22.57	42.41	16.69	57.41	12.67	67.67
EML 53	20.35	19.75	2.95	17.58	13.61	15.54	23.64
EML 57	27.27	26.54	2.68	24.54	10.01	21.21	22.22
AVL13056	35.93	33.24	7.49	30.25	15.81	27.558	23.3
AVL13119	41.27	39.54	4.19	35.54	13.88	33.25	19.43
AVM11207	45.35	33.85	25.36	30.55	32.64	19.19	57.68
CHT119075	35.35	22.65	35.93	17.27	51.15	11.41	67.72
CM 140	27.05	16.69	38.3	16.11	40.44	14.69	45.69
I 162	34.55	24.95	27.79	17.15	50.36	9.27	73.17
I 168	44.57	41.25	7.45	39.54	11.29	35.54	20.26
I 169	66.55	60.52	9.06	55.24	16.99	52.21	21.55
I 170	31.55	29.19	7.48	25.54	19.05	22.23	29.54
I 171	36.15	31.09	14	30.61	15.33	22.65	37.34

Genotypes	At control	At 3 days water logging	% reduction	At 6 days waterlogging	% reduction	At 9 days water logging	% reduction
I 172	51.65	48.65	5.81	46.35	10.26	41.55	19.55
I 185	56.95	50.21	11.83	48.554	14.74	42.52	25.34
I 189	40.15	33.45	16.69	28.35	29.39	22.09	44.98
I 192	31.75	29.54	6.96	26.65	16.06	20.21	36.35
I 200	38.55	21.85	43.32	18.55	51.88	13.45	65.11
I 204	32.65	29.19	10.6	22.65	30.63	20.65	36.75
I 205	38.75	37.27	3.82	27.15	29.94	21.19	45.32
I 206	42.35	27.61	34.81	22.35	47.23	14.65	65.41
JCY 20	46.75	42.21	9.71	40.25	13.9	35.65	23.74
LM 12	54.55	35.19	35.49	22.81	58.19	15.85	70.94
LM 13	44.35	33.27	24.98	23.19	47.71	21.75	50.96
LM 16	19.35	15.29	20.98	13.27	31.42	12.17	37.11
LM 17	37.27	30.65	17.76	28.65	23.13	26.75	28.23
LM 19	27.35	20.11	26.47	16.65	39.12	12.57	54.04
LM 20	38.55	23.49	39.07	13.01	66.25	11.19	70.97
LM 22	28.55	27.19	4.76	22.55	21.02	15.54	45.57
LM 23	36.35	25.55	29.71	18.41	49.35	16.21	55.41
LM 26	18.75	17.85	4.8	15.29	18.45	12.41	33.81
LM 5	32.61	27.19	16.62	15.75	51.7	12.57	61.45
LM 6	27.19	25.54	6.07	22.22	18.28	17.58	35.34
PML 1012	27.19	20.41	24.94	17.27	36.48	13.19	51.49
PML 1228	39.55	36.65	7.33	30.25	23.51	28.32	28.39
PML 1229	33.55	21.27	36.6	12.662	62.26	9.41	71.95
PML 1241	29.15	24.81	14.89	19.95	31.56	6.85	76.5
PML 169	39.27	26.61	32.24	18.79	52.15	14.57	62.9
PML 224	41.15	26.63	35.29	22.55	45.2	18.35	55.41
PML 243	31.19	29.32	6	25.63	17.83	21.23	31.93
PML 28	39.19	34.65	11.58	32.55	16.94	24.95	36.34
PML 368	40.07	36.65	8.54	32.21	19.62	29.23	27.05
PML 373	20.75	17.15	17.35	16.65	19.76	14.21	31.52
PML 387	35.27	31.02	12.05	29.35	16.78	24.45	30.68
PML 400	32.75	30.65	6.41	28.63	12.58	20.3	38.02
PML 898	30.55	22.65	25.86	16.65	45.5	12.21	60.03
SE 503	36.65	32.57	11.13	29.25	20.19	19.25	47.48
SE 616	24.15	17.49	27.58	15.61	35.36	12.57	47.95
Z. mays spp parviglumis	32.15	30.12	6.31	29.32	8.8	27.85	13.37
VL-1010764	33.35	28.65	14.09	25.65	23.09	23.32	30.07

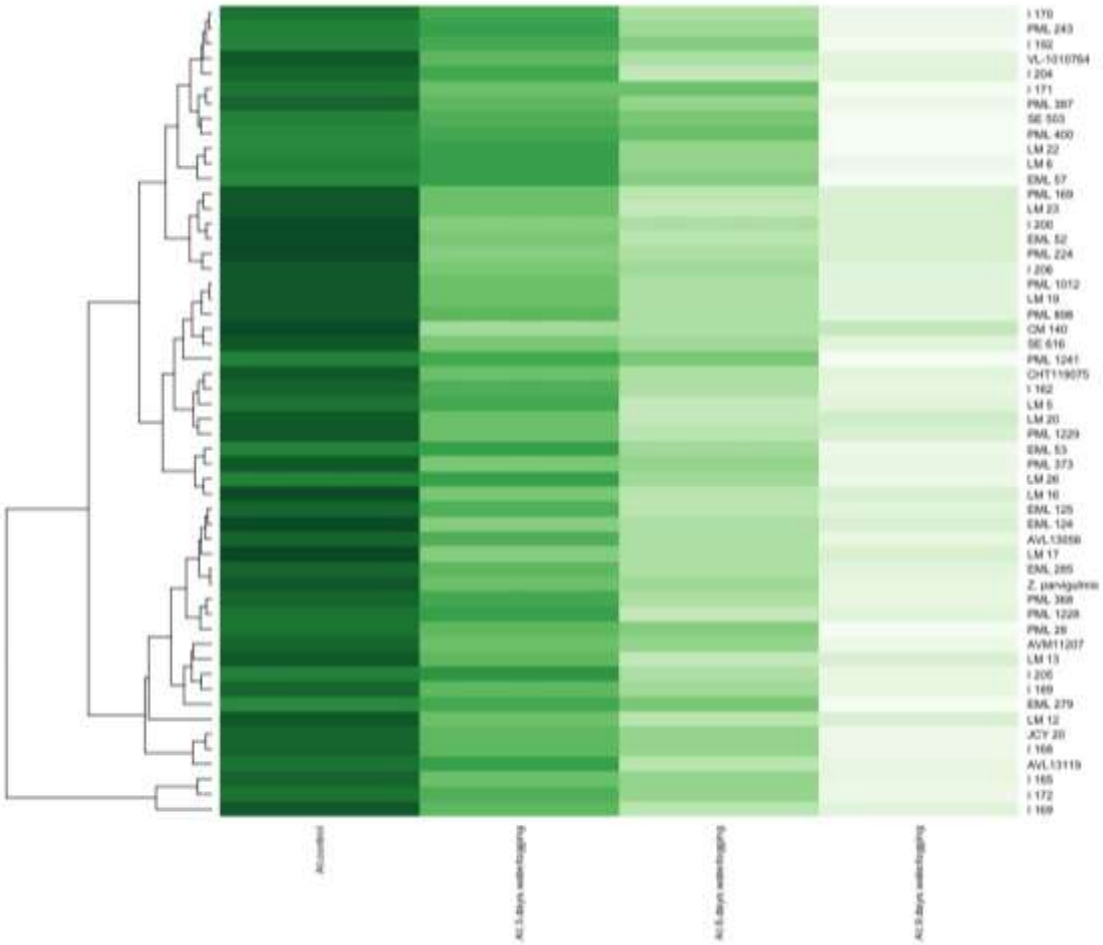


Fig. 4.3: Heatmap showing change in chlorophyll content at different duration of waterlogging.

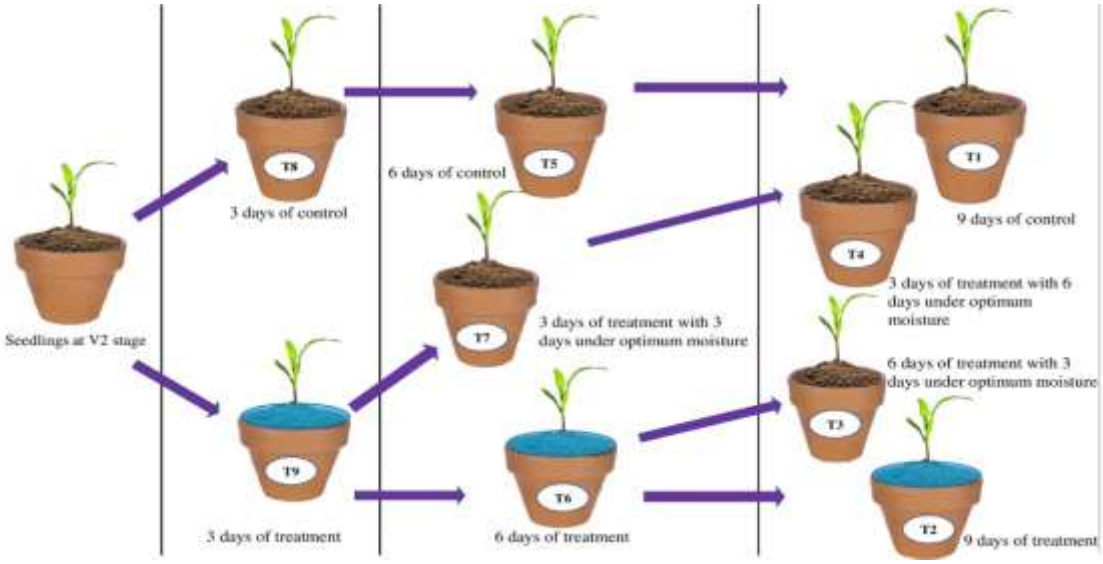


Fig. 4.4: Schematic presentation of different treatments of waterlogging at seedling stage under artificial pit condition.

4.2.3.1 Seedling fresh weight (grams)

Plants were removed from each of the subsequent treatments and one seedling from each pot was harvested and fresh weight was recorded with the help of standard weighing balance. All the values were recorded in grams. Shoot fresh weight significantly reduced under waterlogging stress and 9 days of continuous waterlogging had the most severe effect over fresh weight. At the respective stages, roots were harvested from each pot and washed with bucket of water. The dirt was gently washed off and dried with the tissue paper. These roots were weighed with an aid of weighing balance and data recorded in grams. Root fresh weight significantly reduced under waterlogging stress and 9 days of continuous waterlogging had the most severe effect over fresh weight. Data recorded over different duration of waterlogging were presented in Table 4.15. At all duration of waterlogging, seedlings fresh weight decreased as compared to control. But a significant difference was observed for lines when the duration of stress was extended to 9 days. Some lines had drastic decline in the fresh weight while the change was minimal for other lines. The highest reduction in the shoot fresh weight was as high as 90.14% in PML 1241, while the least was for I 172 with just 2.79% reduction. For root, the highest reduction was seen for LM 19 (87.31%) and least was for I 192 (11.97%). Lines with per cent reduction higher than 75% for both the shoot and root fresh weight after 9 days of waterlogging includes EML 279, AVM11207, I 162, I 189, I 205, I 206, LM 19, LM 23, LM 5, PML 1012, PML 1229, PML 1241, PML 400 and PML 898. Lines with percent reduction less than 25% for both root and shoot fresh weight includes EML 125, EML 285 and I 185. The schematic representation of different duration of waterlogging is shown in Fig. 4.4. Trend of change in shoot fresh mass and root fresh mass are shown in Fig. 4.5 and Fig. 4.6 respectively.

4.2.3.2 Seedling dry weight (grams)

The shoot and root samples harvested for fresh weight determination were allowed to dry over hot air oven at 80^{o} C for 72 hours and dry weight was measured with the help of standard weighing balance. Data was recorded in grams. Shoot dry weight similar to fresh weight significantly reduced under waterlogging stress and 9 days of continuous waterlogging had the most severe effect over dry weight. Root dry weight also showed similar response as fresh weight and got significantly reduced under waterlogging stress and 9 days of continuous waterlogging had the most severe effect over dry weight. The change in root dry weight at different interval of waterlogging is presented in Table 4.16. Difference in percent dry mass reduction became significantly evident when the duration of waterlogging was extended to 9

days. Under nine days of waterlogging highest percentage reduction on dry weight (shoot basis) was observed for PML 898 with 88.88% reduction, while lowest was for I 185 (15.11%). For roots, highest reduction was for I 206 (93.50%) and lowest reduction was recorded for I 185 (15.23%). Lines with per cent reduction higher than 75% for both the shoot and root dry weight after 9 days of waterlogging includes EML 279, I 206, LM 19, LM 20, PML 224 and PML 898. A single line I 185 showed percent reduction less than 25% for both root and shoot fresh weight. Change in shoot and root dry mass in different interval of waterlogging are shown in Fig. 4.7 and Fig. 4.8 respectively.

4.2.3.3 Shoot length (cm)

The ength of fresh shoot samples and root samples were measured with the help of measuring rular and data recorded in cm. The shoot length was measured between the root-shoot junction and tip of the longest leaf. Root length was measured between the root-shoot junction and tip of the longest root. Shoot length got drastically reduced over waterlogging stress and 9 days of waterlogging caused severe decline in shoot length. Similar to shoot length, the root length also showed declining trend over increasing duration of waterlogging. Compared to control, 9 days of waterlogging severely affected the root length. At nine days of waterlogging the highest percentage decline in shoot length was observed in PML 898 (57.62%) compared to control at 9 days while the minimum was recorded for EML 53 (6.06%). For root length, the highest and lowest decline was recorded for PML 1229 (61.29%) and LM 17 (13.34%). Lines showing severe decline (>50%) in both root and shoot length after 9 days of waterlogging were AVM11207 and PML 1229, while the lines with least decline (<20%) in shoot and root length were EML 53, AVL13056, I 172, LM 17 and *Zea mays spp parviglumis*. Shoot and root length across different interval of waterlogging is presented in Table 4.17. Change in shoot and root length across different interval of waterlogging is shown in Fig. 4.9 and Fig. 4.10.

4.2.3.4 Root and Shoot related parameters

Root and shoot samples were harvested from nine different growth environment as mentioned earlier and collected separately. Freshly harvested roots were then collected in falcon tube containing 70% ethanol and stored until further root and shoot analysis was carried out. The roots were scanned in EPSON Expression 12000XL scanner and analyzed using Biovis PSM root software. The software yielded several parameters such as projected total area, total length of the root, number of forks and segments, minimum, maximum and average root diameter and root volume. For shoot area, they were scanned using EPSON

Expression 12000XL scanner and analyzed using Biovis PSM shoot software. The software yielded total shoot area and expressed in square cm. The results showed all three parameters *viz.*, the shoot area, root area and root volume were severely affected with waterlogging.

Similar to other phenotypic observations, shoot and root parameters also showed the maximum difference after nine days of waterlogging and under waterlogging all the root and shoot parameters drastically declined. LM 12 showed the greatest decline (85.48%) in root area under 9 days of waterlogging as compared to control plant. While the minimum decline was for EML 53 (14.11%). For root volume, maximum and minimum reduction was exhibited by PML 1012 (96.22%) and VL-1010764 (24.69%) respectively. Lines showing higher reduction (>70%) for both root area and volume were I 162, I 189, I 205, I 206, LM 12, LM 19, LM 20, PML 1012, PML 1229, PML 1241, PML 169 and PML 224. Lines with low reduction (<50%) for both percentage root area and volume includes EML 57, I 168, I 172 and LM 6.

Shoot area revealed the similar trend as root area and showed the maximum decline at 9 days of waterlogging. The most severely affected genotype was PML 1241 with 94.47% decline in shoot area. Minimum effect on shoot area was for I 185 (26.07%). The lines with percentage reduction higher than 80% were EML 279, EML 52, AVM 11207, I 162, I 189, I 200, I 205, I 206, LM 12, LM 13, LM 19, PML 1241, PML 224, PML 400 and PML 898. Lines with minimum reduction in shoot area (<50%) was seen in I 172, I 185, LM 26 and LM 6.

There was a drastic decline in shoot and root parameters in response to waterlogging and observations recorded for each parameters under different intervals of waterlogging are presented in Table 4.18 and Table 4.19. Scanned images of root and shoot across different interval of waterlogging analysed for one tolerant line, I 172 and one susceptible line, PML 400 is presented in Plate 4.2 and Plate 4.3 respectively. These images showed that over different interval of waterlogging, tolerant lines were able to overcome the ill effects and maintain normal morphological parameters similar to control, but the susceptible lines had drastic decline in overall parameters over different intervals of waterlogging. Trend in change of root and shoot related parameters , *viz.* shoot area, root area and root volume are presented in Fig. 4.11, Fig. 4.12 and Fig. 4.13 respecitively.

Based on comparative study of morphological characters in different duration of waterlogging, it became quite evident that waterlogging stress causes significant reduction in different root and shoot measurable parameters. But depending on the genotypes, different level of tolerance is exhibited. The tolerant lines had minimal reduction in values for various

morphological parameters, but the susceptible lines showed drastic decline in these values. Based on the percentage change in morphological parameters, susceptible and tolerant lines could be clustered into sub-groups with varying level of tolerance.

4.2.3.5 Two-way ANOVA for recorded physiological seedling characteristics:

Data for all the parameters observed at seedling stage were recorded in three replications and observed readings were subjected to two way ANOVA analysis using R studio. All the nine different physiological parameters studied at seedling stage showed no significant variation for replications but for other remaining sources of variation, *viz.*, genotypes, treatments and genotypes : treatment interaction, significant differences were observed. ANOVA table for SFW is shown in Table 4.5 , RFW in Table 4.6, SDW in Table 4.7, RDW in Table 4.8, SL in Table 4.9, RL in Table 4.10, RA in Table 4.11, RV in Table 4.12, and SA in Table 4.13.

Table 4.5: Two way ANOVA for SFW

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	0.17	0.08	0.3859	0.6801
Genotypes	53	1161.03	21.91	101.78	<2e-16*
Treatments	3	1073.61	357.87	1662.7247	<2e-16*
Genotypes*Treatments	159	765.35	4.81	22.3646	<2e-16*
Residuals	430	92.55	0.22		

*** = significant at 0% probability level**

Table 4.6: Two way ANOVA for RFW

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	0.19	0.09	0.4489	0.6386
Genotypes	53	1131.06	21.34	103.0566	<2e-16*
Treatments	3	1070.1	356.7	1722.5383	<2e-16*
Genotypes*Treatments	159	700.69	4.41	21.2813	<2e-16*
Residuals	430	89.04	0.21		

*** = significant at 0% probability level**

Table 4.7: Two way ANOVA for SDW

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	0.029	0.015	2.0337	0.1321
Genotypes	53	176.793	3.336	461.3872	<2e-16*
Treatments	3	120.882	40.294	5573.3645	<2e-16*
Genotypes*Treatments	159	59.071	0.372	51.3866	<2e-16*
Residuals	430	3.109	0.007		

*** = significant at 0% probability level**

Table 4.8: Two way ANOVA for RDW

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	0	0.0002	0.0254	0.9749
Genotypes	53	44.856	0.8463	116.2974	<2e-16*
Treatments	3	24.152	8.0505	1106.228	<2e-16*
Genotypes*Treatments	159	12.543	0.0789	10.8401	<2e-16*
Residuals	430	3.129	0.0073		

*** = significant at 0% probability level**

Table 4.9: Two way ANOVA for SL

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	1.2	0.6	0.0645	0.9376
Genotypes	53	11383.3	214.8	22.4848	<2e-16*
Treatments	3	20847.6	6949.2	727.4996	<2e-16*
Genotypes*Treatments	159	13285.3	83.6	8.7473	<2e-16*
Residuals	430	4107.4	9.6		

*** = significant at 0% probability level**

Table 4.10: Two way ANOVA for RL

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	29.1	14.53	1.2424	0.2897
Genotypes	53	7743.2	146.1	12.4905	<2e-16*
Treatments	3	8815.2	2938.4	251.2152	<2e-16*
Genotypes*Treatments	159	5566.5	35.01	2.9931	<2e-16*
Residuals	430	5029.6	11.7		

*** = significant at 0% probability level**

Table 4.11: Two way ANOVA for RA

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	29	14	1.1996	0.3023
Genotypes	53	155451	2933	245.6482	<2e-16*
Treatments	3	370474	123491	10342.6895	<2e-16*
Genotypes*Treatments	159	143737	904	75.7124	<2e-16*
Residuals	430	5134	12		

*** = significant at 0% probability level**

Table 4.12: Two way ANOVA for RV

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	58	29	1.7507	0.1749
Genotypes	53	921132	17380	1053.6819	<2e-16*
Treatments	3	528829	176276	10687.0433	<2e-16*
Genotypes*Treatments	159	515064	3239	196.3935	<2e-16*
Residuals	430	7093	16		

*** = significant at 0% probability level**

Table 4.13: Two wayANOVA for SA

Source of Variation	*df*	*SS*	*MS*	*f value*	*p-value*
Replications	2	17	9	0.3038	0.7382
Genotypes	53	155226	2929	104.2219	<2e-16*
Treatments	3	1325317	441772	15720.6239	<2e-16*
Genotypes*Treatments	159	265373	1669	59.3925	<2e-16*
Residuals	430	12084	28		

*** = significant at 0% probability level**

4.2.3.6 Correlation study between WTC of different physiological parameters

To elucidate the presence of relationship (if any) between studied physiological parameters, WTC of the each nine traits *viz.*, SFW, RFW, SDW, RDW, SL, RL, SA, RA and RV under study calculated based on the mean value recorded for different duration of waterlogging were subjected to Pearson correlation study in SPSS version 25. The analysis showed highest correlation between shoot area (SA) and root length (RL) and lowest was between shoot length (SL) and shoot dry weight (SDW). The results from the study is presented in Table 4.14.

Table 4.14: Correlation analysis between different waterlogging tolerance coefficient (WTC) of SFW, RFW, SDW, RDW, SL, RL, SA, RA and RV

Correlations

	WTC_SFW	*WTC_RFW*	*WTC_SDW*	*WTC_RDW*	*WTC_SL*	*WTC_RL*	*WTC_SA*	*WTC_RA*	*WTC_RV*
WTC_SFW	1								
WTC_RFW	.742*	1							
WTC_SDW	0.032	-0.077	1						
WTC_RDW	0.134	0.184	-0.076	1					
WTC_SL	.628*	.628*	.392*	0.14	1				
WTC_RL	.783*	.846*	-0.133	0.108	.667*	1			
WTC_SA	.848*	.820*	-0.084	0.069	.655*	.861*	1		
WTC_RA	.727*	.774*	-0.202	0.116	.534*	.769*	.813*	1	
WTC_RV	0.041	0.091	0.207	.408*	0.078	0.02	-0.096	-0.008	1

*** Correlation is significant at the 0.01 level.**

4.3 Selection indices

A number of phenotypic traits were included for the current study. Traits considered for the analysis were shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), shoot area (SA), root

(RA) and root volume (RV). Each of these traits have equal share to predict the tolerant nature of the genotype and thus were included in our study. Two linear selection indices theory, namely Base Linear Phenotypic Selection Index (BLPSI) and Eigen Selection Index Method (ESIM) were used for the current study. For BLPSI, we assigned an economic weightage of one for each of the traits considered, while ESIM assigned the weightage based on eigen value. Since both the approaches considered different value for each trait, some difference was seen in result output. Genotypes selected by both the approaches includes EML 153, SE 503, EML 125, AVL13119, VL-1010764, I 168, I 204, AVL13056 and PML 368. Apart from these genotypes, ESIM approach selected LM 17, *Z. mays spp parviglumis*, PML 373, PML 387, EML 285, JCY 20, I 170, while BLPSI selected I 185, I 172, PML 1228, PML 243, PML 28, EML 124 and LM 6.

Sixteen genotypes based on 30% selection intensity were yielded from each approach assigning LM 17 the highest value as per ESIM index (7.78) and SE 503 the highest value as per BLPSI index (345.58). Genotypes selected from ESIM and BLPSI index were presented in Table 4.20 and Table 4.21 respectively. Asghar and Mehdi (2010) used selection indices for improving yield and quality traits in sweet corn. Sandhu *et al* (2019) reported the use of selection indices in improving the selection efficiency in Indian mustard.

Table 4.15: Seedlings fresh weight (grams) in response different intervals of waterlogging

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
EML 124	5.28	4.98	4.2	3.1	4.71	3.56	4.611	4.23	2.81	1.96	0.39	1.13	2.67	1.28	2.08	1.13	0.29	0.92
EML 125	5.39	6.2	4.18	4.75	4.49	4.87	4.46	5.33	2.99	1.37	2.05	0.79	2.13	0.79	1.99	1.35	1.73	1.09
EML 285	4.36	4.12	3.96	3.24	4.02	3.97	4.36	3.63	1.9	2.3	0.79	1.33	1.08	1.78	0.88	0.23	0.63	0.14
EML 279	7.02	6.25	1.29	1.39	1.75	2.78	2.82	3.33	1.98	2.45	0.77	2.03	1.5	2.17	1.39	0.62	0.42	0.33
EML 52	6.93	5.78	2.17	1.46	2.51	2.94	1.37	2.46	1.98	1.95	1.15	1.25	1.32	1.61	1.13	0.26	0.81	0.23
EML 53	7.56	5.98	5.89	4.21	6.65	4.93	6.56	4.25	2.51	2.17	0.94	1.15	2.3	1.35	1.25	0.1	0.59	0.03
EML 57	5.44	6.23	3.58	5.25	4.05	5.95	4.05	5.79	2.23	2.71	0.41	1.96	1.68	2.63	1.41	1.75	0.23	1.4
AVL13056	7.58	5.1	4.98	4.12	5.1	4.42	6.9	4.56	3.69	1.9	0.39	1.56	3.52	1.68	1.68	0.76	0.23	0.58
AVL13119	4.5	9.52	2.87	7.89	3.01	7.93	3.6	8.13	3.42	3.35	0.2	2.59	1.25	3.18	0.86	0.49	0.28	0.32
AVM11207	9.65	8.7	1.57	0.99	3.24	1.76	3.6	4.08	1.37	2.29	0.64	1.69	1.16	1.8	0.81	0.3	0.43	0.15
CHT119075	5.87	5.54	2.25	1.26	3.21	2.94	4.56	4.44	2.85	1.97	1.48	1.49	2.83	1.94	2.42	0.29	1.15	0.15
CM 140	7.11	6.32	1.01	2.01	1.36	2.22	2.12	2.5	1.59	1.69	0.91	1.22	1.32	1.63	1.14	0.73	0.61	0.58
I 162	7.74	6.98	2.18	1.21	2.68	2.21	3.15	3.54	1.75	2.37	1.21	1.53	1.72	2.03	1.72	0.7	0.93	0.38
I 168	9.95	5.98	7.58	3.95	7.9	4.12	8.9	4.53	4.31	1.35	1.41	0.96	1.69	1.28	1.29	0.53	1.01	0.31
I 169	5.58	5.41	4.12	4.08	4.98	4.12	4.27	4.98	2.13	2.29	1.23	1.74	1.88	1.97	1.68	0.88	0.88	0.46
I 170	4.01	8.55	3.04	6.24	3.57	7.33	3.98	7.1	1.55	3.91	0.39	2.91	0.6	3.9	0.57	1.03	0.17	0.57
I 171	6.2	5.58	1.24	2.52	1.63	3.07	2.66	4.42	1.45	2.63	0.28	1.83	1.4	2.23	0.41	0.95	0.16	0.47
I 172	5.01	7.8	4.87	5.01	5.01	5.12	4.95	5.79	1.85	2.78	1.5	2.05	1.71	2.77	1.52	1.88	1.07	1.59

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
I 185	4.91	8.23	3.96	7.01	4.07	7.89	3.93	7.08	1.77	3.4	0.8	1.87	1.41	3.39	0.87	0.82	0.6	0.44
I 189	7.97	7.25	2.25	1.79	3.48	2.01	3.12	2.63	1.62	1.66	0.41	1.27	1.61	1.62	1.52	0.64	0.31	0.44
I 192	5.65	5.51	4.21	4.85	4.88	4.91	5.1	4.99	1.26	3.24	0.93	2.8	1.22	2.85	0.91	0.7	0.83	0.53
I 200	7.71	6.98	2.56	2.92	4.52	2.98	4.09	3.01	3.01	1.56	1.62	0.78	1.83	1.37	1.74	1.71	1.08	1.03
I 204	4.56	8.12	3.89	5.65	3.96	6.12	3.04	7.47	1.52	2.99	0.68	1.68	0.7	2.79	0.67	1.22	0.47	0.98
I 205	6.27	5.64	1.24	1.01	1.52	1.38	3.78	4.57	1.45	2.8	0.95	2.01	1	2.37	0.91	0.17	0.7	0.07
I 206	8.21	7.84	1.67	1.2	2.38	1.53	3.19	6.53	1.44	3.15	0.69	2.05	1.33	2.91	0.55	0.99	0.47	0.6
JCY 20	8.51	4.21	5.87	3.12	6.01	3.25	6.14	4.1	3.12	2.13	2.33	1.09	2.96	1.07	2.77	1.12	1.55	0.62
LM 12	6.96	5.93	1.57	2.28	1.71	2.82	4.22	3.33	1.77	1.95	1.53	1.55	1.62	1.64	1.56	1.35	1.36	0.87
LM 13	6.98	5.98	2.21	1.43	2.41	2.33	3.3	4.05	1.43	1.49	1.28	1.14	1.41	1.16	1.4	0.46	0.94	0.3
LM 16	6.34	5.65	4.01	4.1	4.88	4.51	5.21	4.88	1.49	2.42	0.75	2.21	0.98	2.31	0.98	1.7	0.82	1.13
LM 17	4.25	4.85	3.14	3.95	3.24	4.12	3.56	4.28	1.91	1.91	1.06	1.62	1.48	1.78	0.97	0.29	0.64	0.27
LM 19	8.54	8.12	0.99	1.03	1.93	2.02	1.41	1.59	1.13	0.77	0.58	0.4	0.67	0.42	0.37	0.51	0.3	0.3
LM 20	6.47	5.65	1.74	2.19	2.7	3.96	1.96	2.88	1.99	1.84	0.61	1.48	1.57	1.57	1.47	0.99	0.42	0.6
LM 22	6.78	5.69	2.17	2.11	2.95	2.36	3.53	4.85	1.85	1.97	1.09	1.68	1.6	1.93	1.43	0.18	0.53	0.08
LM 23	8.54	8.45	1.83	1.64	4.9	3.75	2.22	3.67	2.95	1.65	1.15	1.05	1.51	1.71	1.15	0.47	1.35	0.34
LM 26	6.1	5.54	4.25	4.08	4.87	4.92	5.1	4.22	1.27	2.47	0.63	1.32	1.03	1.52	0.92	0.29	0.38	0.2
LM 5	6.8	5.75	1.5	1.33	5.14	3.65	3.01	3.25	1.95	2.29	1.21	1.46	1.22	1.73	1.22	0.34	0.8	0.24
LM 6	5.43	6.23	4.56	4.21	4.86	5.12	4.98	5.52	3.14	3.31	0.76	2.3	3.02	2.5	2.95	0.97	0.37	0.63
PML 1012	6.98	5.98	1.67	1.21	1.71	1.41	5.03	3.08	1.95	1.72	1.04	1.09	1.87	1.14	1.18	0.42	1.01	0.21

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
PML 1228	5.12	7.12	3.98	5.32	4.1	5.98	4.78	6	1.17	2.33	0.36	1.45	0.73	1.82	0.55	0.93	0.22	0.62
PML 1229	7.12	6.65	1.54	0.98	1.82	1.06	3.09	2.33	1.31	1.17	1.12	0.68	1.14	0.88	0.96	0.46	0.67	0.19
PML 1241	10.25	9.25	1.01	1.95	1.75	2.25	1.58	3.62	2.96	2.8	0.8	1.94	2.93	2.18	2.75	0.66	0.23	0.49
PML 169	5.69	5.51	2.1	1.2	2.56	3.6	2.3	2.97	2.14	1.37	1.46	0.85	1.96	0.9	1.92	0.35	0.93	0.16
PML 224	7.25	6.98	2.26	1.19	2.67	1.28	2.85	1.76	1.11	0.86	0.315	0.55	0.83	0.67	0.73	1.03	0.2	0.82
PML 243	5.12	4.82	4.12	2.68	4.82	3.12	5.1	4.12	2.58	2.36	0.94	1.18	1.94	1.94	0.93	0.92	0.53	0.63
PML 28	5.25	4.87	4.85	3.12	4.9	3.85	5.1	4.1	2.58	2.85	1.96	1.91	2.49	2.54	2.35	1.06	1.4	0.69
PML 368	9.64	8.1	6.87	5.65	7.21	6.55	8.41	7.25	3.19	3.46	0.59	2.06	2.96	3.23	1.56	1.57	0.55	1.31
PML 373	9.001	6.95	7.21	5.17	8.07	6.1	8.42	6.32	3.32	2.38	1.78	1.35	1.9	1.96	1.47	1.36	1.14	1.14
PML 387	6.45	5.65	4.02	4.25	5.74	4.65	5.15	5.16	2.78	2.55	1.21	1.37	2.71	2.29	2.3	0.66	0.71	0.55
PML 400	10.25	9.54	2.6	1.77	3.49	2.41	4.74	5.15	1.44	1.77	0.88	1.2	1.23	1.33	0.96	0.83	0.84	0.49
PML 898	10.4	10.2	1.64	2.56	2.66	4.01	1.95	3.98	0.85	4.23	0.45	2.33	0.53	3.02	0.53	0.59	0.29	0.45
SE 503	4.95	4.28	3.12	3.21	4.55	3.25	3.72	3.65	1.98	1.94	1.56	1.26	1.77	1.45	1.43	1.19	1.02	0.92
SE 616	5.89	5.54	3.98	4.25	4.1	4.42	4.28	5.13	1.62	2.18	0.41	1.64	1.58	1.97	0.91	0.97	0.17	0.61
Z. mays spp parviglumis	8.05	7.23	5.12	5.65	6.52	6.65	5.87	6.15	2.95	3.22	1.25	2.28	1.38	3.04	1.39	0.35	1.16	0.29
VL-1010764	4.52	6.38	2.52	4.56	3.22	5.25	3.05	6.1	2.856	3.17	1.62	2.05	1.91	2.32	1.75	1.36	1.14	0.74

T1: 9 days of control, T2: 9 days of waterlogging, T3: 6 days of waterlogging followed by 3 days of optimum moisture, T4: 3 days of waterlogging followed by 6 days of optimum moisture, T5: 6 days of control, T6: 6 days of waterlogging, T7: 3 days of waterlogging followed by 3 days of optimum moisture, T8: 3 days of control, T9: 3 days of waterlogging

Table 4.16: Seedlings dry weight (grams) as observed in different interval of waterlogging

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
EML 124	1.98	1.56	0.98	0.99	1.1	1.25	1.84	1.35	1	0.86	0.83	0.63	0.93	0.73	0.17	0.17	0.11	0.1
EML 125	2.97	0.96	1.25	0.44	1.58	0.46	2.11	0.69	1.19	0.41	0.81	0.29	1.12	0.37	0.1	0.22	0.06	0.21
EML 285	1.91	0.98	1.01	0.56	1.13	0.57	1.61	0.65	0.64	0.32	0.55	0.22	0.59	0.28	0.33	0.14	0.28	0.13
EML 279	1.64	0.45	0.25	0.09	0.35	0.27	0.88	0.42	0.7	0.17	0.55	0.07	0.63	0.17	0.1	0.04	0.09	0.04
EML 52	1.88	0.45	0.41	0.13	0.52	0.35	0.4	0.2	0.83	0.25	0.56	0.22	0.79	0.25	0.17	0.06	0.08	0.03
EML 53	2.89	1.85	1.25	1	1.56	1.27	2.23	1.19	0.9	0.65	0.5	0.45	0.86	0.57	0.47	0.06	0.39	0.04
EML 57	2.12	0.96	1.75	0.54	1.95	0.65	2.01	0.8	0.87	0.43	0.66	0.38	0.82	0.4	0.23	0.13	0.18	0.11
AVL13056	3.21	1.24	2.12	0.91	2.24	1.05	2.44	1.07	1.19	0.45	0.88	0.3	0.99	0.34	0.36	0.11	0.21	0.04
AVL13119	1.4	1.25	0.85	0.85	0.92	1.16	0.92	1.02	0.54	0.57	0.29	0.51	0.51	0.5	0.07	0.07	0.05	0.06
AVM11207	1.85	0.58	0.52	0.12	1.32	0.49	1.48	0.44	0.7	0.29	0.5	0.24	0.57	0.29	0.21	0.03	0.12	0.02
CHT119075	1.89	0.85	0.48	0.12	0.61	0.38	1.7	0.71	0.83	0.35	0.71	0.29	0.78	0.34	0.19	0.04	0.11	0.03
CM 140	1.91	1.01	0.91	0.18	1.09	0.33	0.98	0.29	0.62	0.37	0.35	0.17	0.49	0.32	0.33	0.09	0.2	0.07
I 162	1.64	0.93	0.68	0.24	1.21	0.41	1.58	0.64	0.75	0.44	0.36	0.28	0.66	0.44	0.31	0.06	0.35	0.05
I 168	4	0.63	2.55	0.44	3.21	0.45	3.38	0.56	1.81	0.24	1	0.12	1.75	0.19	0.57	0.16	0.33	0.13
I 169	1.88	0.85	1.29	0.34	1.69	0.51	1.42	0.41	0.67	0.36	0.38	0.23	0.48	0.3	0.54	0.09	0.34	0.07
I 170	2.98	1.45	1.89	0.78	2.15	1.2	2.1	1	1.17	0.64	0.81	0.4	0.91	0.61	0.28	0.07	0.15	0.05
I 171	1.68	0.86	0.67	0.16	0.91	0.64	0.87	0.49	0.81	0.3	0.6	0.28	0.6	0.28	0.25	0.07	0.2	0.06
I 172	2.18	0.88	1.19	0.62	1.48	0.63	1.39	0.72	0.93	0.38	0.85	0.27	0.86	0.34	0.51	0.13	0.26	0.09

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
I 185	2.25	1.05	1.91	0.89	2.1	0.93	1.98	1	0.98	0.37	0.55	0.26	0.66	0.28	0.43	0.08	0.26	0.05
I 189	1.49	0.54	0.94	0.11	1.42	0.18	1.06	0.29	0.59	0.25	0.33	0.18	0.71	0.23	0.38	0.11	0.2	0.07
I 192	1.89	0.94	1.21	0.44	1.35	0.43	1.43	0.61	0.83	0.37	0.58	0.28	0.73	0.31	0.35	0.14	0.28	0.12
I 200	0.98	0.74	0.54	0.34	0.85	0.52	0.62	0.49	0.34	0.31	0.29	0.2	0.31	0.31	0.26	0.14	0.17	0.14
I 204	1.84	1.49	1.25	1.1	1.58	1.4	1.57	1.23	0.7	0.66	0.52	0.39	0.6	0.48	0.09	0.06	0.06	0.05
I 205	2.29	0.61	0.72	0.15	0.96	0.22	1.28	0.58	0.84	0.39	0.52	0.26	0.56	0.32	0.26	0.04	0.23	0.03
I 206	1.98	1.54	0.29	0.1	1.03	1.19	0.95	0.85	0.71	0.75	0.65	0.54	0.67	0.7	0.11	0.04	0.08	0.03
JCY 20	2.87	0.95	1.21	0.44	2.01	0.75	2.07	0.46	1.41	0.43	0.99	0.23	1.13	0.43	0.34	0.08	0.29	0.08
LM 12	2.12	0.75	0.84	0.19	1.04	0.37	1.99	0.37	1.02	0.32	0.82	0.25	0.89	0.26	0.36	0.07	0.23	0.06
LM 13	2.11	0.65	1.01	0.31	1.12	0.46	1.25	0.42	1.06	0.3	0.85	0.14	0.85	0.25	0.36	0.14	0.26	0.12
LM 16	2.03	0.82	1.25	0.45	1.59	0.71	1.29	0.54	0.88	0.42	0.63	0.29	0.63	0.35	0.43	0.06	0.22	0.05
LM 17	1.95	0.98	1.1	0.65	1.34	0.75	1.16	0.85	0.7	0.39	0.53	0.22	0.61	0.32	0.43	0.03	0.28	0.01
LM 19	4.25	0.45	0.87	0.06	1.31	0.37	3.75	0.26	1.83	0.27	1.45	0.17	1.67	0.23	0.42	0.02	0.33	0.02
LM 20	2.63	1.54	0.59	0.25	1.32	0.8	0.81	0.33	1.34	0.92	1.07	0.54	1.24	0.83	0.21	0.11	0.12	0.08
LM 22	2.66	0.98	1.01	0.3	1.09	0.67	1.67	0.73	1.23	0.36	0.78	0.2	0.76	0.34	0.34	0.13	0.33	0.05
LM 23	2.55	1.54	0.89	0.14	2.01	0.69	0.9	0.4	1.02	0.52	0.6	0.29	0.75	0.34	0.36	0.04	0.18	0.03
LM 26	1.69	0.92	0.88	0.58	0.92	0.88	1.39	0.75	0.84	0.32	0.49	0.18	0.53	0.23	0.27	0.05	0.11	0.03
LM 5	2.05	0.85	0.82	0.08	1.71	0.52	0.87	0.33	0.57	0.31	0.44	0.22	0.48	0.17	0.36	0.09	0.3	0.08
LM 6	1.82	1.08	1.1	0.74	1.7	0.87	1.8	0.89	0.78	0.4	0.44	0.24	0.66	0.36	0.32	0.15	0.27	0.14
PML 1012	2.65	1.65	0.81	0.3	1.14	0.36	2.4	0.91	1.23	0.6	1.06	0.46	1.05	0.51	0.29	0.18	0.16	0.02

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
PML 1228	2.36	1.08	1.25	0.55	1.29	0.75	1.45	0.82	0.76	0.41	0.52	0.27	0.69	0.3	0.31	0.07	0.23	0.04
PML 1229	1.32	0.97	0.63	0.11	1	0.12	0.77	0.52	0.56	0.35	0.42	0.25	0.55	0.3	0.25	0.06	0.22	0.04
PML 1241	2.11	0.65	0.64	0.19	1.33	0.26	0.89	0.38	0.78	0.26	0.57	0.17	0.73	0.22	0.34	0.14	0.22	0.13
PML 169	1.75	0.46	0.71	0.12	0.92	0.13	0.83	0.38	0.76	0.22	0.47	0.09	0.52	0.1	0.3	0.03	0.26	0.01
PML 224	1.84	0.48	0.39	0.08	1.05	0.33	1.3	0.1	0.91	0.3	0.75	0.13	0.89	0.28	0.17	0.06	0.1	0.02
PML 243	2.11	0.87	0.85	0.65	1.1	0.74	1.64	0.71	1.02	0.4	0.55	0.27	0.86	0.37	0.24	0.06	0.19	0.04
PML 28	1.92	0.65	0.99	0.45	1.01	0.48	1.52	0.49	0.84	0.26	0.69	0.18	0.78	0.19	0.35	0.09	0.25	0.05
PML 368	3.98	1.45	2.1	0.98	2.68	1.1	3.04	1.09	1.36	0.53	0.9	0.29	1.23	0.27	0.5	0.17	0.35	0.16
PML 373	2.98	0.85	1.93	0.4	2.14	0.58	2.64	0.72	1.23	0.34	0.73	0.23	1.09	0.19	0.13	0.24	0.05	0.22
PML 387	2.85	0.91	1.85	0.52	2.03	0.61	2.62	0.56	1.13	0.4	0.99	0.26	1.14	0.36	0.37	0.06	0.3	0.01
PML 400	1.89	0.97	0.87	0.25	1.48	0.47	1.42	0.6	0.93	0.52	0.51	0.24	0.78	0.49	0.41	0.11	0.3	0.08
PML 898	3.69	1.14	0.41	0.18	0.89	0.42	1.12	0.5	1.33	0.37	1.07	0.2	1.13	0.33	0.2	0.08	0.11	0.04
SE 503	2.82	0.93	1.69	0.44	2.05	0.54	1.99	0.8	1.01	0.4	0.75	0.19	0.91	0.29	0.28	0.16	0.24	0.08
SE 616	2.25	0.85	1.68	0.65	1.71	0.75	1.85	0.75	1.05	0.38	0.67	0.2	0.89	0.24	1	0.04	0.12	0.04
Z. mays spp parviglumis	2.1	0.86	1.02	0.64	1.95	0.78	1.78	0.71	0.97	0.32	0.66	0.21	0.74	0.27	0.24	0.08	0.16	0.02
VL-1010764	2.21	1.02	1.85	0.56	2.1	0.74	1.96	0.76	0.89	0.35	0.63	0.21	0.78	0.24	0.31	0.12	0.13	0.07

T1: 9 days of control, T2: 9 days of waterlogging, T3: 6 days of waterlogging followed by 3 days of optimum moisture, T4: 3 days of waterlogging followed by 6 days of optimum moisture, T5: 6 days of control, T6: 6 days of waterlogging, T7: 3 days of waterlogging followed by 3 days of optimum moisture, T8: 3 days of control, T9: 3 days of waterlogging

Table 4.17: Seedling length (cm) as measured across different intervals of waterlogging

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
EML 124	40	27	29	16	32	18	39	21	30	17	28	15	28	16	16	12	15	10
EML 125	36	29	28	22	31	23	35	24	28	28	24	21	26	27	21	9	18	8
EML 285	35	27	25	19	27	23	35	24	29	21	23	17	27	19	11	14	9	13
EML 279	47	30	21	19	25	22	37	20	24	22	20	18	23	19	20	17	17	15
EML 52	45	29	25	18	28	23	31	24	26	24	24	17	24	19	20	8	17	8
EML 53	33	21	31	18	32	20	32	20	35	20	31	15	32	16	12	13	11	11
EML 57	40	28	27	19	30	20	37	23	30	20	25	14	28	20	14	11	12	11
AVL13056	37	25	34	20	34	21	35	23	34	24	32	18	32	21	15	12	13	12
AVL13119	36	23	29	16	30	17	34	20	26	17	21	15	21	15	24	7	23	7
AVM11207	49	33	24	15	26	18	36	19	29	20	24	14	25	20	15	12	13	10
CHT119075	40	28	27	15	28	19	39	20	33	19	27	14	28	15	19	9	16	8
CM 140	48	31	22	17	26	18	32	27	26	23	22	16	26	21	11	7	10	6
I 162	48	32	35	21	38	22	39	25	41	22	34	20	34	22	15	12	13	10
I 168	37	24	34	17	36	18	32	16	33	20	31	14	33	19	17	12	15	11
I 169	40	28	32	22	36	23	29	23	25	26	24	21	25	25	13	11	12	10
I 170	37	25	31	19	34	20	35	22	26	24	22	17	24	18	14	11	13	10
I 171	42	28	24	16	29	21	37	20	27	19	22	13	26	14	16	13	15	13
I 172	38	29	33	23	36	26	32	27	34	23	32	20	34	22	17	15	16	14

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
I 185	52	26	46	19	48	21	38	24	44	24	42	18	44	21	19	14	17	13
I 189	48	32	39	16	40	19	41	13	39	20	38	15	38	16	20	10	17	9
I 192	40	28	30	18	32	22	33	19	29	23	26	16	28	18	15	8	14	7
I 200	48	31	24	19	26	20	28	22	24	22	23	17	24	18	13	8	12	8
I 204	40	28	33	19	35	20	37	21	35	22	32	17	34	18	11	13	10	12
I 205	42	29	23	16	24	17	33	21	26	17	21	15	22	17	16	7	15	7
I 206	48	32	26	18	27	19	32	20	29	20	24	17	26	18	14	13	13	11
JCY 20	41	23	26	18	28	21	36	19	31	22	25	16	29	19	16	16	14	15
LM 12	45	29	30	14	32	15	35	18	32	19	29	13	32	18	14	11	13	10
LM 13	45	30	25	21	26	22	32	26	22	26	20	20	20	24	12	12	11	10
LM 16	44	29	32	21	33	26	42	25	31	22	29	20	31	21	19	10	16	9
LM 17	34	23	29	20	31	21	29	22	31	25	28	19	28	19	15	16	14	14
LM 19	48	32	34	12	36	18	43	19	35	15	33	11	35	14	17	9	15	8
LM 20	42	29	18	22	25	24	29	24	23	25	19	20	23	23	13	12	11	11
LM 22	44	29	29	19	34	21	44	22	32	20	27	18	29	19	17	13	15	12
LM 23	49	32	26	15	29	18	33	18	26	15	24	13	25	15	20	11	19	9
LM 26	41	28	32	19	32	20	32	21	27	20	24	18	27	18	12	11	11	9
LM 5	45	29	25	19	26	24	27	25	25	22	23	18	24	20	15	11	14	11
LM 6	40	27	26	18	28	20	32	19	24	23	23	17	24	21	17	12	15	10
PML 1012	46	30	32	17	34	23	38	24	31	22	29	15	29	19	17	13	15	11

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
PML 1228	38	26	29	19	31	20	35	23	28	19	23	17	23	19	15	10	14	8
PML 1229	48	31	21	12	22	20	22	14	22	15	20	11	21	12	13	8	12	7
PML 1241	49	35	25	23	26	23	31	25	28	25	23	22	23	25	16	13	14	12
PML 169	40	28	21	16	28	19	34	19	23	19	19	15	21	17	16	13	15	11
PML 224	48	31	23	18	25	21	26	25	29	23	24	17	27	18	21	9	19	9
PML 243	51	36	46	23	48	27	49	29	49	28	44	22	49	26	17	11	15	9
PML 28	39	27	25	20	29	21	31	22	27	24	23	17	24	20	14	14	12	12
PML 368	42	25	38	16	40	21	41	22	36	16	32	12	33	15	23	12	19	11
PML 373	38	25	17	18	24	20	32	21	30	19	25	17	28	18	15	15	14	14
PML 387	42	35	36	22	37	29	39	28	39	28	36	20	39	25	16	12	15	10
PML 400	56	38	24	21	26	23	38	24	26	26	22	19	26	22	27	13	24	12
PML 898	59	42	25	25	26	26	45	29	28	29	24	24	24	25	17	11	15	10
SE 503	35	27	27	21	29	25	34	26	29	23	26	20	27	23	24	9	21	8
SE 616	41	28	26	18	30	28	36	24	29	22	25	16	27	20	10	16	9	15
Z. mays spp parviglumis	41	35	33	30	35	32	36	35	33	36	31	29	33	29	14	7	12	6
VL-1010764	36	23	29	18	30	20	31	21	30	22	28	16	29	17	23	10	20	8

T1: 9 days of control, T2: 9 days of waterlogging, T3: 6 days of waterlogging followed by 3 days of optimum moisture, T4: 3 days of waterlogging followed by 6 days of optimum moisture, T5: 6 days of control, T6: 6 days of waterlogging, T7: 3 days of waterlogging followed by 3 days of optimum moisture, T8: 3 days of control, T9: 3 days of waterlogging.

Table 4.18: Root area (square cm) and root volume (cubic cm) at different interval of waterlogging

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL
EML 124	83.2	158	36.4	50.9	42.7	78.8	68.5	106	36.5	50.6	31.7	46.4	35.1	48.5	37.8	24.1	34.4	22.9
EML 125	92.5	190	61.7	30.2	65.6	65.5	86.6	173	48.4	59.8	38.7	54.3	44.7	54.3	19.4	11.4	11.9	9.7
EML 285	58.9	40.2	20.2	18.2	49.8	36.5	45.9	22.6	23.5	13.7	14.3	12.6	20.6	13.1	8.8	9.5	5.4	8
EML 279	98.5	25.6	33.8	8.5	43.5	17.9	20.5	9.3	31.9	7.9	26.3	7.2	31.4	7.3	24	3.9	13.3	3.7
EML 52	98.3	80.2	17.6	33.5	20.6	56.9	32.1	62.8	14	28.6	8.2	26.3	11.2	27.5	31.6	13.3	18.9	11.9
EML 53	40.5	282	34.8	110	35	242	37.1	28.4	43.3	80.5	37.7	70	40.2	74	45.6	4.9	27.6	4.3
EML 57	95.7	80.2	60	52.8	88	75.9	39.9	61.1	56.9	23.5	41.5	21.4	48.2	22.8	41.5	19.9	38.4	17.8
AVL13056	108.9	289	47.1	34.1	54.5	208	67.5	190	83.1	77.9	46.2	68.3	82.5	70.6	38.9	14.5	25.8	12.4
AVL13119	79.9	96.8	34.9	21.4	55.9	45.4	74	58.8	47.5	31.2	37.7	29.5	37.9	29.8	30.6	8.5	21.7	7.5
AVM11207	118.7	52.8	21.6	19.5	58.1	28.7	22.3	31.2	33	15.1	18.2	13.4	24.4	13.7	29.2	9	20.9	8.5
CHT119075	87.9	89.6	26.5	20.4	65.5	68.1	44.8	31.1	43.2	23.7	24.5	20.4	27.5	21.2	29.1	7.9	20.8	7.2
CM 140	98.5	45.5	21.3	15.9	32	18.7	67.2	38	56	12	49.5	11.3	50.4	11.8	16.7	6.9	11.4	6.5
I 162	103.1	59.5	20.8	5.8	36.9	32.2	46.1	32.7	38.2	19.8	36.4	17.1	37.4	17.3	47.2	2.1	34.2	1.9
I 168	166.2	154	121	85	63.1	94.8	117.6	98.2	69.3	41.6	37.1	35.9	49.5	36.7	29.1	5	24.7	4.3
I 169	85.6	49.5	26.8	18.4	38.7	23.5	35.2	39.2	16.5	17.6	8.8	15.1	13.2	16.2	32.2	8.3	17.5	7.3
I 170	68.9	30.5	31.5	9.4	46.4	28.3	34.3	10.4	57.5	9.7	36.4	9	52.3	9.6	10.7	4.3	6	3.6
I 171	91.5	45.8	34.3	28	70.4	40.4	42	34	39.1	14.9	21.4	13.7	23.2	14	59	10	39.1	8.3
I 172	79.5	112	49.2	65.5	53.2	96.5	58.5	102	31.3	29.7	21.7	27.3	27.9	29.5	52.2	32.3	31.2	30.1

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL
I 185	126.5	181	58.2	44.3	91.9	77	83.3	116	62.5	51.8	40.8	48.9	59.2	50	51.2	16.1	38.5	13.6
I 189	104.4	105	25.8	14.2	35.4	48.6	78.8	96	38.7	27.2	24.7	25.1	38.2	25.4	30.4	5.7	17.6	4.9
I 192	87.2	50.2	39.4	14.6	54.4	44	83.4	46.9	43.4	16.7	24	14.9	27.8	15.2	15.1	6.8	12.5	5.8
I 200	102.5	165	21.6	49.8	36.5	152	40.5	56.5	22.7	21.7	17.4	19.7	21.4	21	38.5	20.1	24.5	18.2
I 204	135.6	92.5	42.8	35.5	89.2	80.5	54.8	44.2	46.4	25.7	43	22.8	46.2	24.5	11.9	16.4	6.6	14.2
I 205	93.3	140	25.8	14.7	75	127	82.4	121	61.3	37.2	55.7	31.5	55.9	34.1	28.9	5.7	16.4	5.1
I 206	105.6	98.5	24.2	11.6	73	93	48	58.9	47.1	31.5	35.1	27.9	40.3	30.4	21.6	5.7	15.5	5.2
JCY 20	85.6	154	44.5	56.1	50.3	76.5	78.3	128	46	46.5	39.6	39.7	45.1	40.7	22.2	10.2	15.3	9.2
LM 12	98.5	72.5	14.3	6.5	45.3	54	43	39.2	26	22.7	24.1	21.4	24.7	22.3	16.3	2.6	11.1	2.5
LM 13	98.5	120	29.8	20.3	51.6	106	39.8	33.8	27.6	39	14.6	36.1	22.8	37.6	25.8	7.7	13.8	7.4
LM 16	93.5	98.6	25.3	47.8	53.7	83.2	34.9	65.8	37	31	19.7	28.7	31.2	31	51.7	21.6	45.8	18.3
LM 17	128.3	85.2	59.6	38.6	57.2	68.2	35.7	28.3	41.1	29.9	31.1	26	39.9	29.7	8.5	6.5	4.7	5.9
LM 19	106.8	60.8	19.7	6.2	24.8	27.3	26.3	38.2	14.1	18.6	10.6	16.3	13.9	16.9	13	3.3	9.3	2.9
LM 20	94.7	92.5	25.9	10.8	46.1	25.9	47	28.3	38.9	31.9	20.9	30.4	25.5	31.5	29.9	5.2	18.5	4.8
LM 22	95.8	87.9	39	25.9	72.2	78.3	48.4	29.3	37.7	28.2	24.1	24.5	37.5	26.8	52.7	10	37.4	8.4
LM 23	109.2	115	52.9	29.2	47.8	39.8	27.1	42	29.1	31.6	22.9	28	28.1	29	36	13.6	29.8	12.5
LM 26	89.8	98.2	36.7	12.8	43.9	34.2	55.4	57.2	37	29	22.8	24.4	33.1	29	28.4	5.5	25.3	5.1
LM 5	96	50.2	20.8	30.3	45	41.2	31.2	38.9	29.6	13.3	23.5	11.2	25.7	13.3	34.4	15.7	24.3	13.6
LM 6	85.1	142	50.4	86.8	78.2	125	59.6	62	31.4	47.1	17.3	44	28.9	44.4	8.7	13.9	5	12.4
PML 1012	98.5	180	28.4	6.8	96.4	159	47.9	31.2	38.8	56	22	52.9	31.7	54.4	43	3.2	23	2.7

Genotypes	T1		T2		T3		T4		T5		T6		T7		T8		T9	
	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL	AREA	VOL
PML 1228	79.6	149	26.2	18.2	61.9	92.2	39.5	22.2	36.1	48.6	26.2	43.8	33.6	48.4	24.9	8.9	17.4	7.8
PML 1229	98.8	89.5	17.4	4.8	56.6	61.7	26.3	9.7	39.6	28.7	24.8	24.4	27.1	26.9	19	1.9	11.7	1.8
PML 1241	125.6	65.8	29.2	11.6	51.2	32.2	30.3	32.6	29.4	22.3	15.9	18.7	21.5	20.1	28.6	4.7	22	4.1
PML 169	87.7	86.6	25.7	8	42.8	21.6	52.7	56	50.2	23.1	32.6	19.8	34.6	20.6	34.5	3.3	21.7	2.9
PML 224	99.6	35.6	21.3	7	26.5	14.8	26.8	9.8	21.7	9.6	17.4	8.1	19.1	8.8	12.1	2.9	10.8	2.7
PML 243	79.8	87.5	46.3	38.1	63.6	76.5	74.6	66.5	61.4	30.3	37.5	28.6	49.5	29	27.5	19.1	22.9	16.6
PML 28	82.6	210	25.1	36.5	82.1	156	39.8	102	34.6	58.9	20.9	53.1	26.9	53.5	23.5	14	19.4	11.7
PML 368	95.8	185	34.7	30.2	80.8	98	42	109	49.2	51.6	43.1	46.5	45.7	47.8	13.6	11.5	8.9	10.7
PML 373	96.6	102	52.8	34	74.5	48.6	71.8	81.2	29.7	29.9	22.8	26.7	27.8	29.6	9.9	13.6	6.9	12.4
PML 387	88.9	140	28.4	36.8	78.4	101	38.6	58.9	45.1	40.6	39.2	36	42.6	38.4	32.4	19.1	22.5	16.6
PML 400	130.5	110	43.4	19.2	58	55.5	50.2	79.6	28.6	33.9	19.1	28.7	24.9	31.9	25.5	8.5	23.4	8.1
PML 898	152.2	58.4	31.2	17.9	55.4	32.1	37.8	29.8	39.3	19.6	30.5	17.6	32.6	17.8	24.4	7.5	14	6.9
SE 503	157.8	196	50.9	44.2	104.5	142	129.8	156	56.6	66.2	34.2	63.1	38	63.5	37.8	22.9	28.1	19.9
SE 616	89.6	65.5	20.4	47.3	70.5	53.4	30.4	59.8	36.1	20.5	27.5	19.5	29.2	20.1	32.3	17.4	22.1	15.2
Z. mays spp parviglumis	135.5	296	38.5	45.4	93.3	206	39.2	42.4	56.3	86.2	36.8	81.3	42.1	83.9	26.5	2.2	18.3	1.8
VL-1010764	165.9	116	65	87.5	67.8	79.4	80.8	95.5	38.5	32.6	35.7	27.4	37.5	29	20.9	9.9	17.3	9.1

T1: 9 days of control, T2: 9 days of waterlogging, T3: 6 days of waterlogging followed by 3 days of optimum moisture, T4: 3 days of waterlogging followed by 6 days of optimum moisture, T5: 6 days of control, T6: 6 days of waterlogging, T7: 3 days of waterlogging followed by 3 days of optimum moisture, T8: 3 days of control, T9: 3 days of waterlogging

Table 4.19: Shoot area (square cm) measured at different interval of waterlogging.

Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	T9
EML 124	120.25	34.14	36.12	116.29	116.23	71.07	103.38	16.37	10.82
EML 125	185.5	28.9	35.33	138.1	76.18	44.24	71.79	9.93	8.31
EML 285	105.85	36.97	49.7	80.03	60.07	52.16	58.07	14.73	8.4
EML 279	145.6	12.38	25.92	85.06	34.06	21.64	32.51	7.97	5.67
EML 52	141.28	16.5	25.54	42.49	33.03	19.11	24	7.23	5.06
EML 53	198.5	26.5	29.14	139.86	80.37	66.23	79.76	12.61	7.07
EML 57	98.7	28.51	36.54	60.04	31.4	19.15	27.27	13.88	9.3
AVL13056	168.9	36.6	43.75	151.97	50.51	32.03	43.01	13.23	8.31
AVL13119	103.2	15.12	33.38	85.5	48.66	31.56	41.03	9.94	6.65
AVM11207	185.5	15.2	28.83	138.56	92.64	63.86	79.62	10.06	7.52
CHT119075	104.25	23.11	35.83	98.24	62.51	37.82	54.08	10.21	8.31
CM 140	150.2	30.2	48.6	69.86	38.2	26.16	34.99	18.37	13.02
I 162	159.52	29.8	32.5	100.79	44.24	32.32	32.56	12.21	9.23
I 168	195.5	59.6	65.25	128.15	53.41	48.55	50.22	38.47	24.81
I 169	98.81	40.57	43.52	50.15	38.96	27.06	33.91	17.57	13.54
I 170	92.15	35.5	47.13	55.67	62.11	39.75	54.12	16.61	9.17
I 171	124.87	25.6	35.21	62.39	22.47	12.33	19.49	10.31	7.3
I 172	68.56	35.9	45.89	60.85	46.24	37.73	44.53	16.89	15.09

Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	T9
I 185	93.2	18.9	23.49	89.5	64.76	44.88	52.18	11.27	7.76
I 189	165.4	32.1	36.31	72.16	45.37	36.76	45.01	15.55	10.84
I 192	98.83	15.2	18.48	85.68	54.56	32.3	36.65	8.72	7.97
I 200	158.6	21.2	25.65	36.89	20.43	12.96	15.06	11.55	8.27
I 204	108.8	19.3	20.21	99.78	49.41	42.03	46.62	10.03	9.18
I 205	133.95	18.2	21.62	74.35	42.32	28.57	39.1	6.83	5.94
I 206	166.29	16.51	31.26	84.64	32.74	26.42	30.61	9.89	5.2
JCY 20	145.5	18.9	24.05	130.6	87.24	47.32	75.32	12.23	6.74
LM 12	141.4	15.5	20.02	73.38	39.31	24.03	35.3	6.14	4.3
LM 13	142.2	14.5	18.99	102.21	81.35	71.47	76.65	9.51	7.56
LM 16	135.5	32.2	42.69	98.88	53.17	48.79	53.04	20.96	15.56
LM 17	137.58	32.1	36.57	106.63	65.19	57.97	65	14.02	11.07
LM 19	169.5	25.8	30.58	40.19	21.05	12.34	13.04	9.85	6.26
LM 20	136.2	29.5	31.11	53.17	71.93	54.4	65.88	13.63	9.85
LM 22	136.6	10.2	21.56	110.75	89.63	66.89	80.21	3.71	3.11
LM 23	170.7	39.23	47.67	63.98	34.62	29	30.3	20.48	12.67
LM 26	112.25	9.2	16.88	90.31	39.08	35.76	37.45	4.63	3.52
LM 5	138.56	28.2	34.42	75.78	61.16	40.61	42.29	9.92	7.55
LM 6	98.6	34.6	43.43	86.61	39.7	28.18	33.74	11.51	6.56

Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	T9
PML 1012	145.5	29.2	35.18	122.04	102.47	69.33	75	10.76	8.57
PML 1228	94.54	23.3	33.42	83.86	35.35	19.36	26	12.75	10.53
PML 1229	152.2	17.2	27.44	74.84	55.29	41.32	43.66	10.25	7.83
PML 1241	185.5	10.25	32.65	60.21	40.18	29.64	33.9	3.91	1.9
PML 169	102.2	25.8	31.2	72.42	63.19	51.16	61.83	12.46	10.9
PML 224	152.2	20.1	21.88	32.65	16.93	10.9	13.42	7.1	6.4
PML 243	135.9	49.2	57.47	116.6	82.26	44.88	71.98	18.62	14.77
PML 28	96.63	29.65	42.56	39.65	20.04	14.86	19.73	17.44	13.01
PML 368	192.58	32.1	36.68	168.07	107.32	60.47	101.4	17.74	14.3
PML 373	152.56	23.25	29.65	146.34	61.7	52.72	52.69	12.81	7.81
PML 387	156.85	41.97	75.85	112.86	67.19	50.6	62.7	23.79	20.92
PML 400	198.5	15.2	31.91	150.85	79.69	44.39	56.57	8.41	4.98
PML 898	198.8	16.35	43.17	23.62	43.81	32.24	34.99	6.21	4.04
SE 503	167.85	41.2	50.5	86.49	34.54	18.29	21.25	25.57	21.59
SE 616	106.24	29.2	31.43	93.87	51.47	45.11	50.56	16.73	14.56
Z. mays spp parviglumis	152.25	48.2	73.3	113.83	58.61	31.69	41.71	17.42	15.02
VL-1010764	85.5	12	23.87	80.03	30.28	19.15	27.81	5.96	3.09

T1: 9 days of control, T2: 9 days of waterlogging, T3: 6 days of waterlogging followed by 3 days of optimum moisture, T4: 3 days of waterlogging followed by 6 days of optimum moisture, T5: 6 days of control, T6: 6 days of waterlogging, T7: 3 days of waterlogging followed by 3 days of optimum moisture, T8: 3 days of control, T9: 3 days of waterlogging

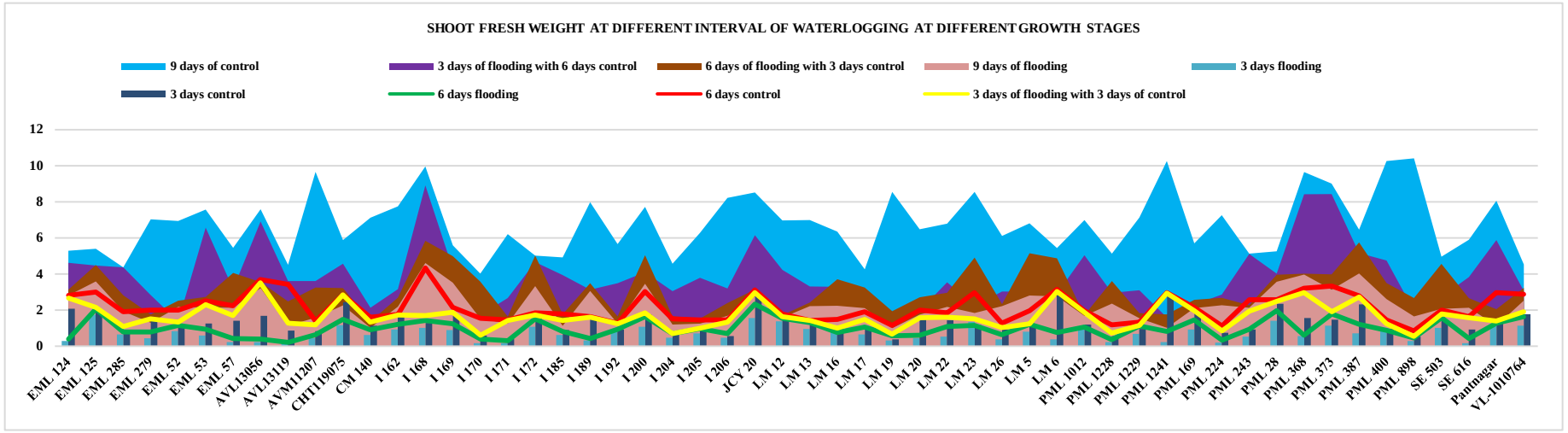


Fig. 4.5: Bar graph showing shoot fresh weight at different interval of waterlogging at different growth stages

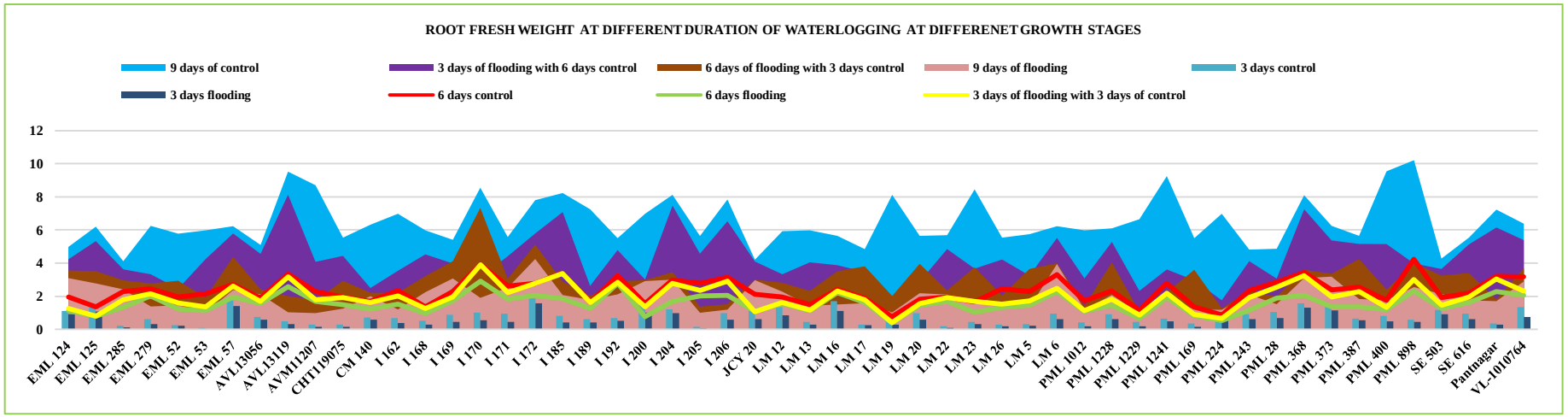


Fig. 4.6: Bar graph showing root fresh weight at different interval of waterlogging at different growth stages

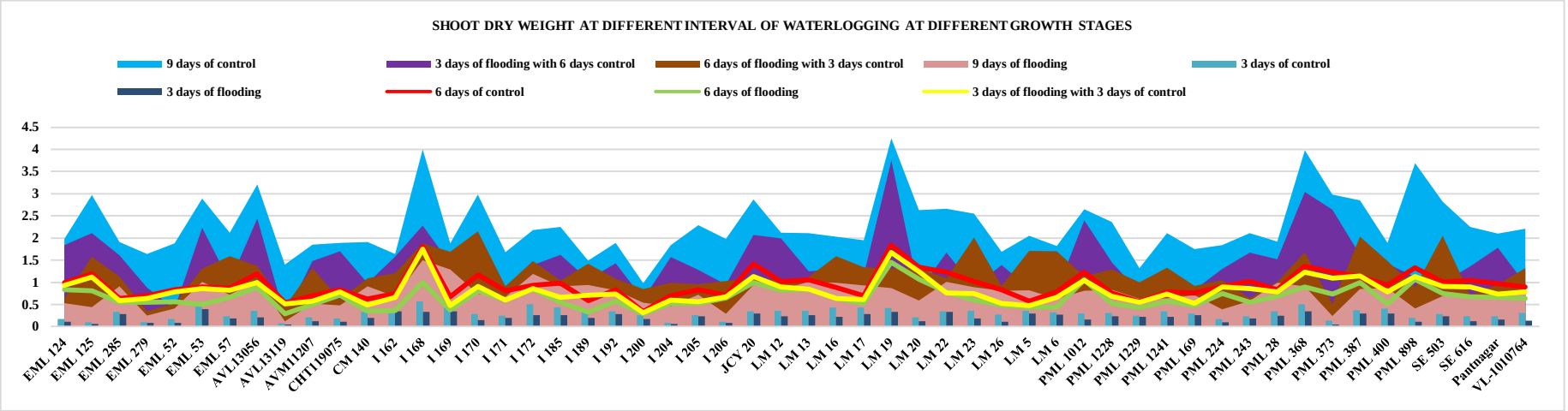


Fig. 4.7: Bar graph showing shoot dry weight at different interval of waterlogging at different growth stages

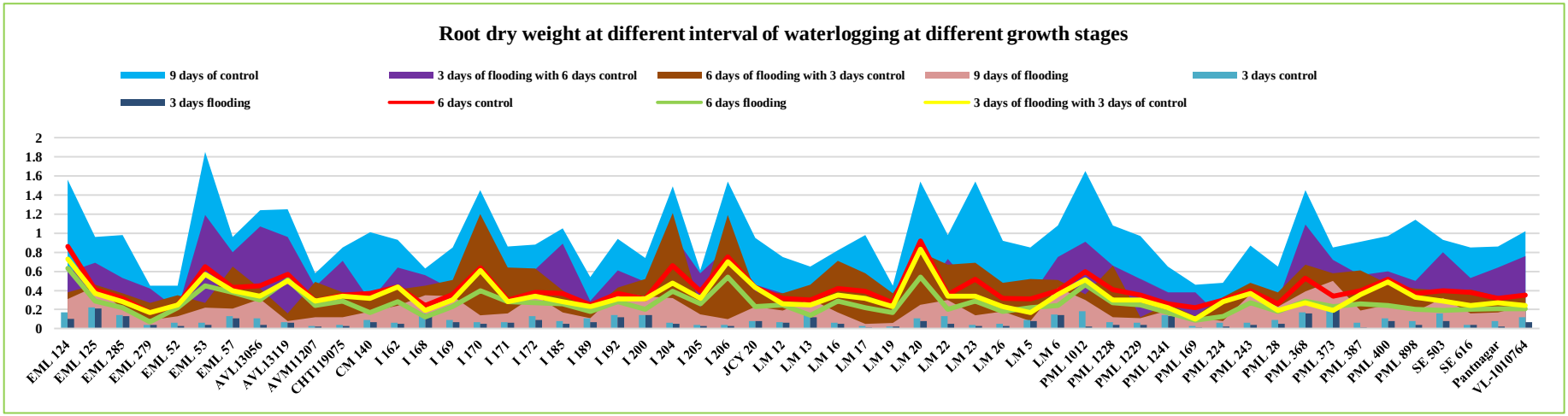


Fig. 4.8: Bar graph showing root dry weight at different interval of waterlogging at different growth stages

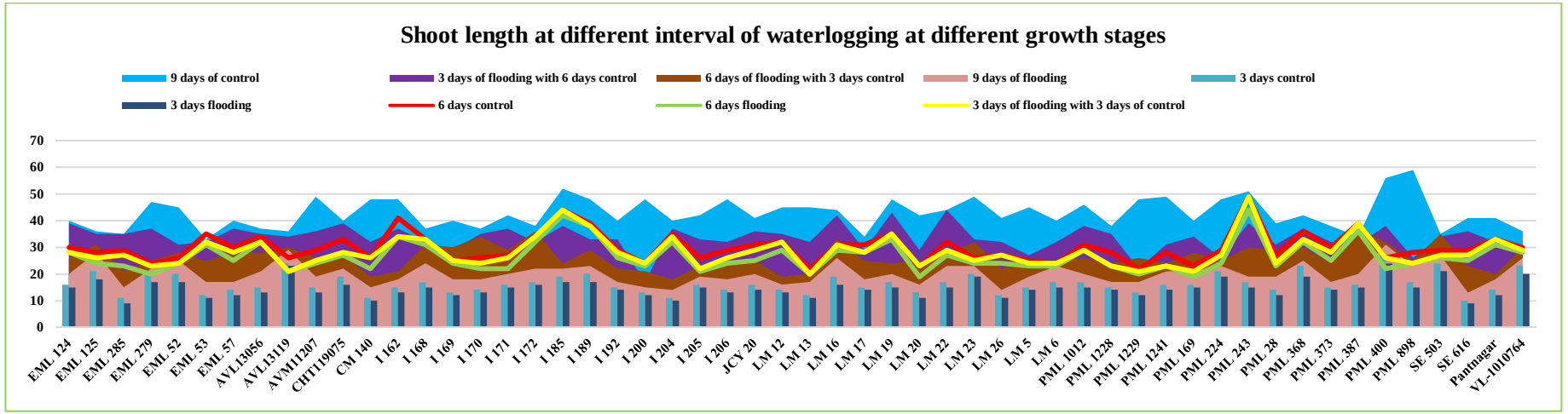


Fig. 4.9: Bar graph showing shoot length at different interval of waterlogging at different growth stages

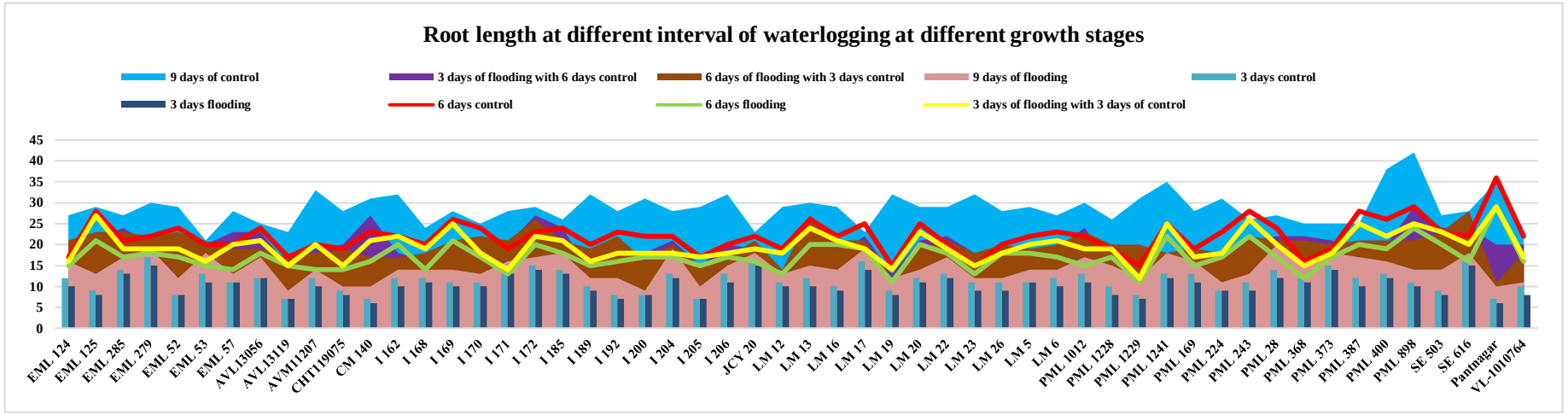


Fig. 4.10: Bar graph showing root length at different interval of waterlogging at different growth stages

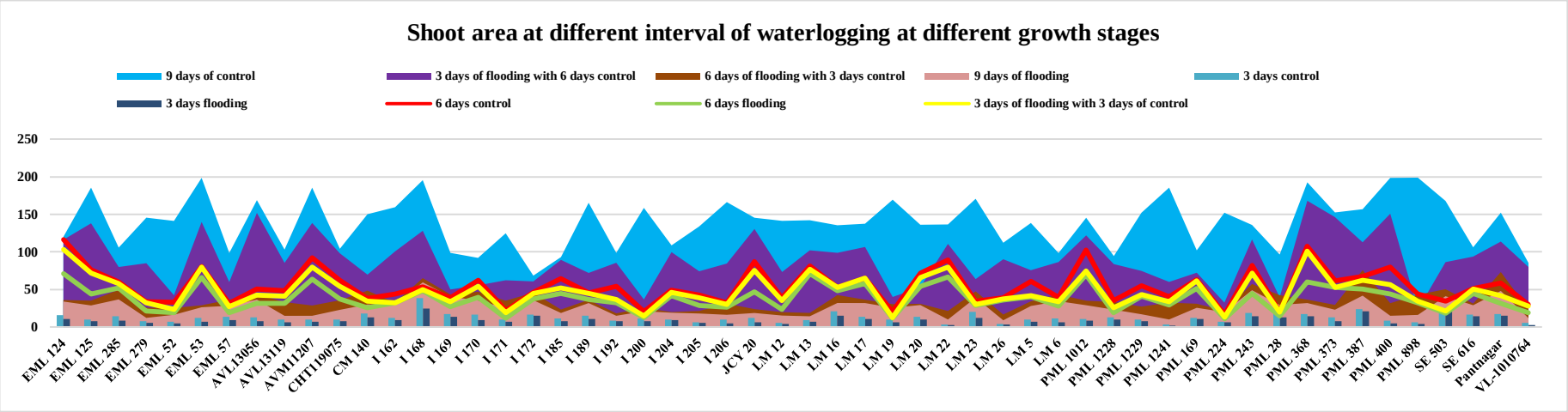


Fig. 4.11: Bar graph showing shoot area at different interval of waterlogging at different growth stages

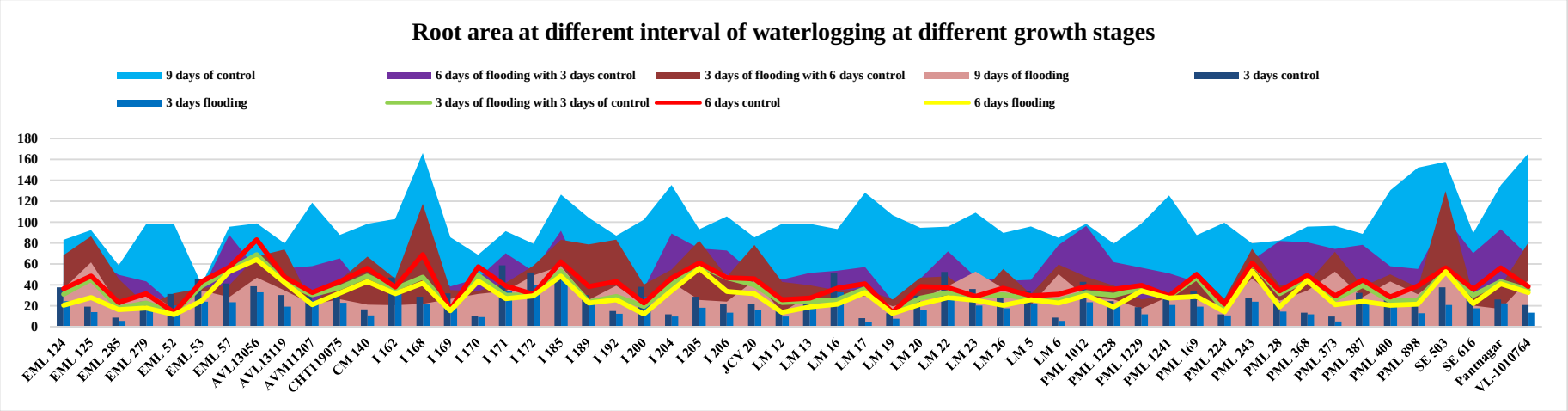


Fig. 4.12: Bar graph showing root area at different interval of waterlogging at different growth stages

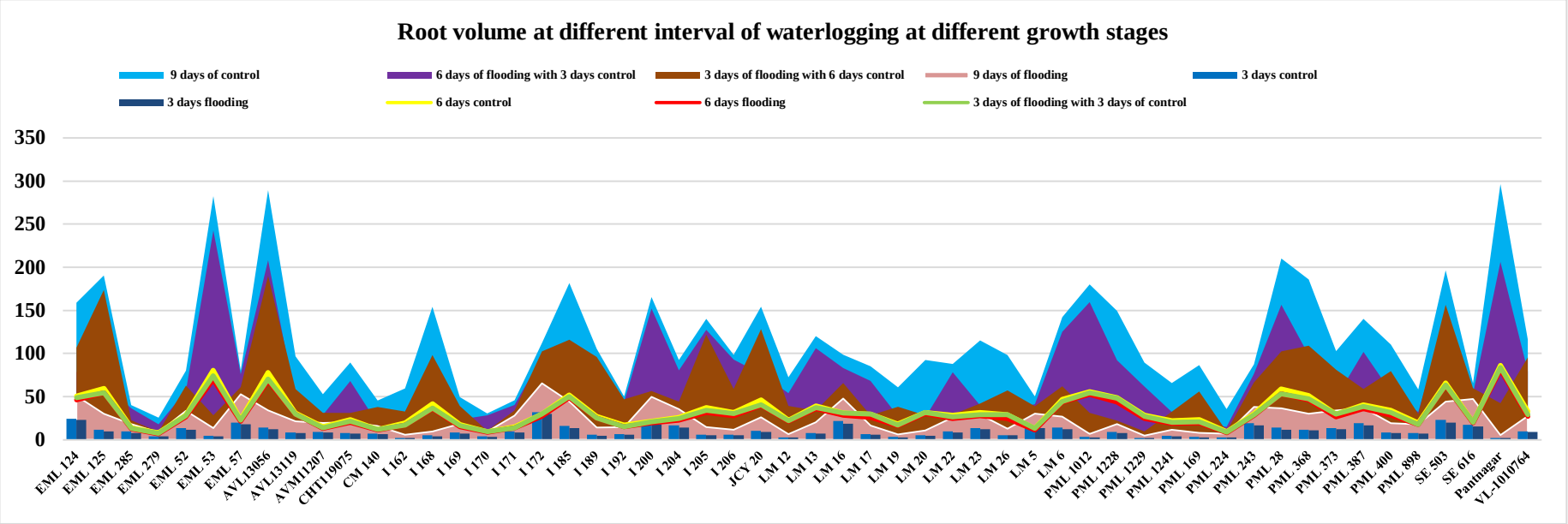


Fig. 4.13: Bar graph showing root volume at different interval of waterlogging at different growth stages

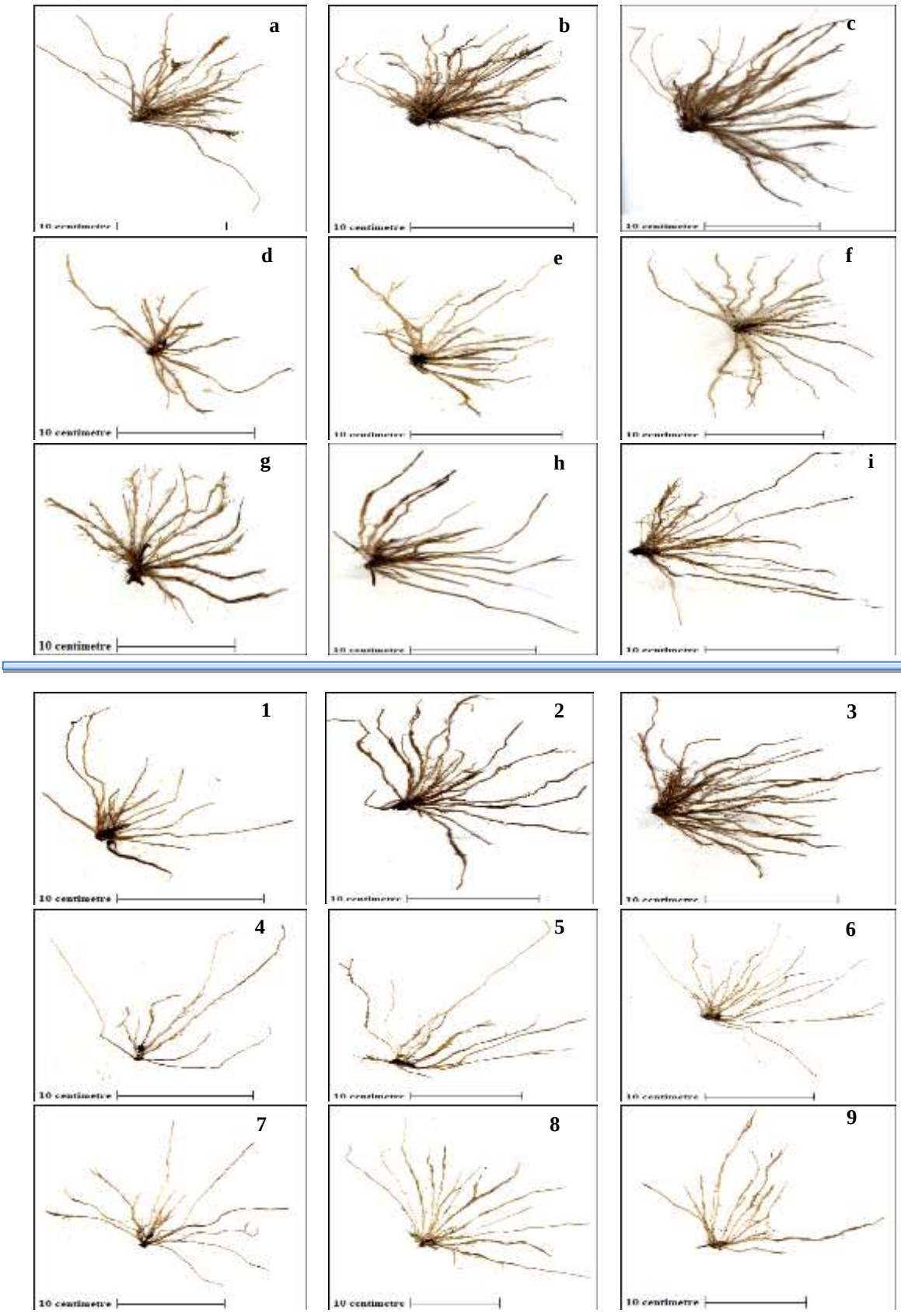


Plate 4.2: Root images of I 172 (a-i) and PML 400 (1-9) at different duration of waterlogging

a,b,c : control at 3,6,9 days; d,e,f : waterlogging for 3,6,9 days; g : 3 days flooding followed by 3 days control, h : 3 days flooding followed by 6 days control; i : 6 days flooding followed by 3 days control

1,2,3 : control at 3,6,9 days; 4,5,6 : waterlogging for 3,6,9 days; 7 : 3 days flooding followed by 3 days control, 8 : 3 days flooding followed by 6 days control; 9 : 6 days flooding followed by 3 days control

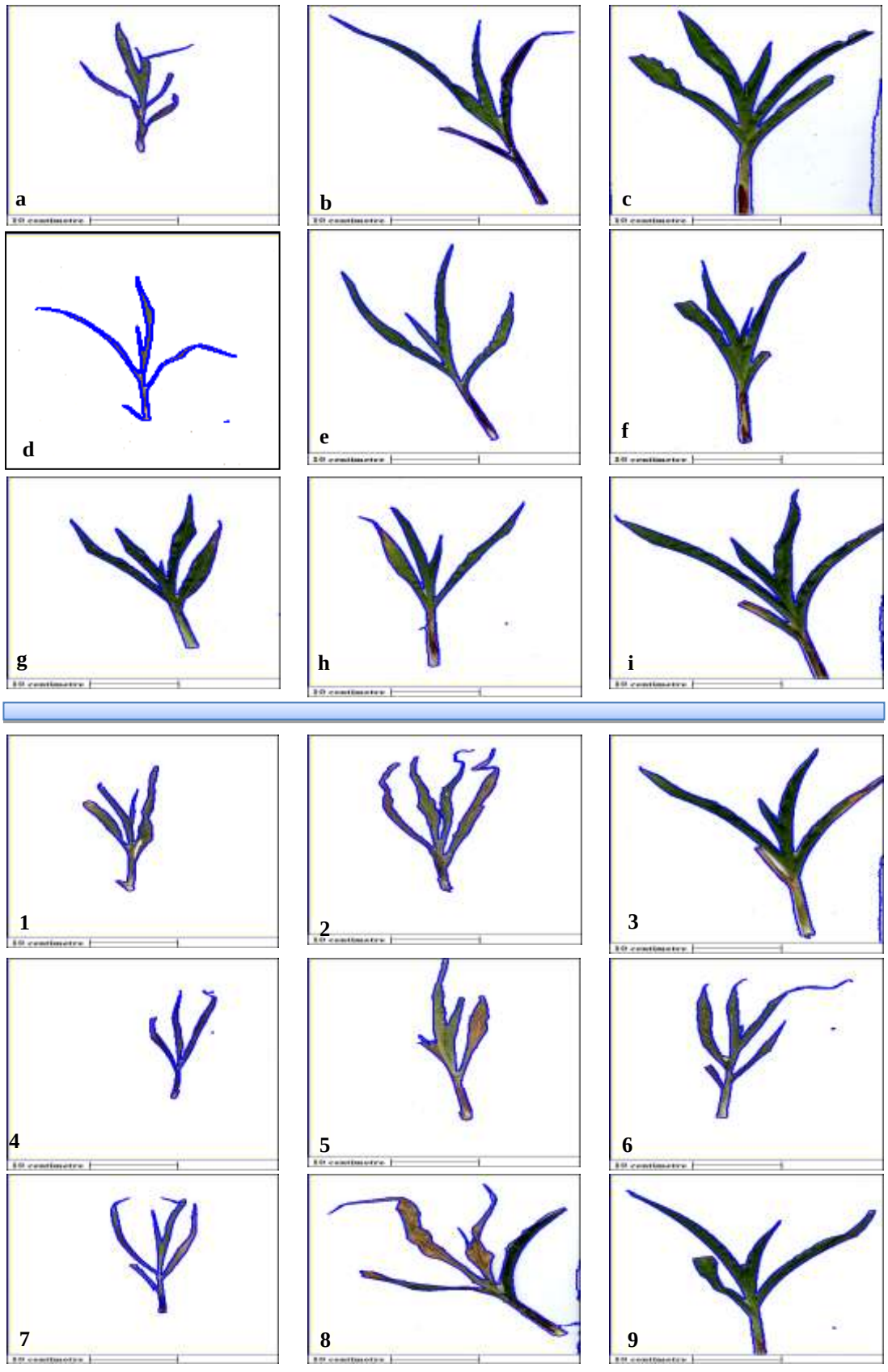


Plate 4.3: Shoot images of I 172 (a-i) and PML 400 (1-9) at different duration of waterlogging

a,b,c : control at 3,6,9 days; d,e,f : waterlogging for 3,6,9 days; g : 3 days flooding followed by 3 days control, h : 3 days flooding followed by 6 days control; i : 6 days flooding followed by 3 days control
1,2,3 : control at 3,6,9 days; 4,5,6 : waterlogging for 3,6,9 days; 7 : 3 days flooding followed by 3 days control, 8 : 3 days flooding followed by 6 days control; 9 : 6 days flooding followed by 3 days control

Table 4.20: List of genotypes selected as per ESIM index

Genotypes	SFW	RFW	SDW	RDW	SL	RL	SA	RA	RV	ESIM index
LM 17	7.24	2.27	4.88	4.29	1.24	1.68	4.84	5	19.86	7.78
Z. mays spp parviglumis	1.79	3.99	1.2	2.07	1.13	1.88	1.93	1.53	11.1	5.5
EML 53	1.98	2.34	5.16	3.23	1.36	1.3	1.52	2.13	17.9	4.95
PML 373	1.16	1.55	3.79	1.62	1.31	0.85	2.81	4.42	15.67	4.81
PML 387	2.11	1.46	1.67	5.91	1.19	1.43	1.85	2.43	10.08	4.19
SE 503	1.28	1.41	0.98	1.22	0.93	1.39	1.39	3.26	17.43	4.16
EML 285	1.22	1.24	1.28	2.35	1.01	1.02	0.88	5.92	13.17	4.03
EML 125	1.32	1.24	2.15	6.08	1.13	1.31	1.62	2.64	11.11	3.95
AVL13119	0.67	0.78	0.24	0.99	0.95	0.64	2.02	2.66	28.73	3.82
JCY 20	1.1	1.42	3.65	1.62	1.02	0.97	1.7	2.42	12.86	3.8
VL-1010764	1.09	1.97	0.9	2.92	0.8	0.98	0.72	2.67	16.3	3.74
I 168	1.56	1.74	1.68	0.63	1.04	0.7	0.97	2.97	17.44	3.7
I 204	1.6	1.14	1.82	1.98	1.36	1.22	1.39	1.9	11.39	3.5
AVL13056	1.2	1.5	1.53	1.93	1.11	1.09	2.2	1.6	5.65	3.44
I 170	0.86	0.89	1.42	0.94	1.27	0.88	1.4	2.87	16.74	3.43
PML 368	1.44	1.53	1.6	3.28	1.14	1.3	1.48	1.5	3.63	3.3
Mean of Selected Individuals	1.73	1.65	2.12	2.57	1.12	1.16	1.8	2.87	14.32	
Mean of all Individuals	1.06	1.06	1.3	2.05	0.94	1.05	1.16	2.01	8.1	
Selection Differential	0.67	0.59	0.82	0.52	0.18	0.12	0.63	0.86	6.22	
Expected Genetic Gain for 30%	0.83	0.44	0.79	0.85	0.13	0.16	0.6	0.76	3.58	

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), shoot area (SA), root (RA) and root volume (RV)

Table 4.21: List of genotypes selected as per BLPSI index

Genotypes	SFW	RFW	SDW	RDW	SL	RL	SA	RA	RV	BLPSI
SE 503	5.62	6.06	1.85	0.72	32.56	21.94	75.19	78.49	132.15	354.58
AVL13056	4.73	4.91	1.47	0.56	32.51	19.23	76.98	69.26	114.25	323.89
I 168	8.85	4.35	2.89	0.72	39.32	21.46	87.11	56.38	100.64	321.72
AVL13119	4.85	3.95	0.63	0.44	29.07	15.69	78.12	40.63	121.63	295
I 185	4.25	5.47	1.49	0.66	40.2	24.14	53.05	72.52	78.78	280.56
VL 1010764	4.82	3.76	1.29	0.49	35.45	19.75	70.38	54.64	79.87	270.46
EML 125	5.4	4.23	2.05	0.43	33.8	22.31	68.16	60.22	69.43	266.02
I 172	4.87	5.59	1.83	0.59	37.01	28.78	59.51	54.68	70.84	263.7
PML 368	5.24	4.45	1.7	0.7	30.98	18.19	74.95	54.14	64.75	255.1
I 204	2.78	4.49	1.16	0.7	30.11	21.39	49.54	57.82	80.4	248.4
EML 53	4.09	3.54	1.28	0.46	30.48	18.01	65.32	51.76	72.62	247.56
PML 1228	4.32	4.28	1.45	0.52	31.57	18.17	60.14	46.61	80.04	247.08
PML 243	4.54	3.49	1.53	0.44	39.94	22.1	76.18	51.86	46.31	246.39
PML 28	4.13	2.26	1.27	0.56	30.82	24.93	40.66	60.95	76.82	242.39
EML 124	4.59	4.41	1.12	0.58	32.3	22.12	62.22	51.08	62.5	240.91
LM 6	4.65	5.51	1.42	0.77	31.89	19.16	63.46	56.34	55.75	238.94
Mean of Selected Individuals	4.86	4.42	1.53	0.58	33.63	21.09	66.31	57.34	81.67	
Mean of all Individuals	4.03	3.57	1.33	0.52	31.66	19.52	56.7	46.79	49.98	
Selection Differential	0.82	0.85	0.2	0.07	1.96	1.56	9.61	10.55	31.69	
Expected Genetic Gain for 30%	1.12	1.11	0.16	0.07	0.96	2.04	11.29	7.58	10.48	

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), shoot area (SA), root (RA) and root volume (RV)

4.4 Multi-trait stability index (MTSI)

Data were collected from four different environment which included seedlings from control plot after 9 days of attaining V_2 stage, seedlings from treatment plot where waterlogging stress was provided for 9 days, seedlings which were at waterlogging condition for 3 days and afterward 6 days under optimal water condition and finally seedlings which were at waterlogging stress for 6 days and afterward 3 days under optimal moisture condition. Nine traits; shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), shoot area (SA), root (RA) and root volume (RV) -were simultaneously measured in all those four environment and most stable and good performing line across all those four environments were measured and genotypes were ranked as per their performance. Selection intensity of 30% was employed to find the best line out of all the genotypes performing best in waterlogging condition. The output is graphically represented in Fig. 4.14.The results from MTSI revealed line I 172 to be most stable and best performing in all four environments. Lines selected as per MTSI were I 172, I 185, LM 6, I 168, I 192, EML 125, SE 503 and SE 616. Three genotypes PML 243, EML 52 and I 169 fell on the border line. Zuffo *et al* (2020) used the MTSI approach to select most stable genotypes in drought and saline condition. Benakanahalli *et al* (2021) used MTSI for identifying the most stable genotypes in Guar.

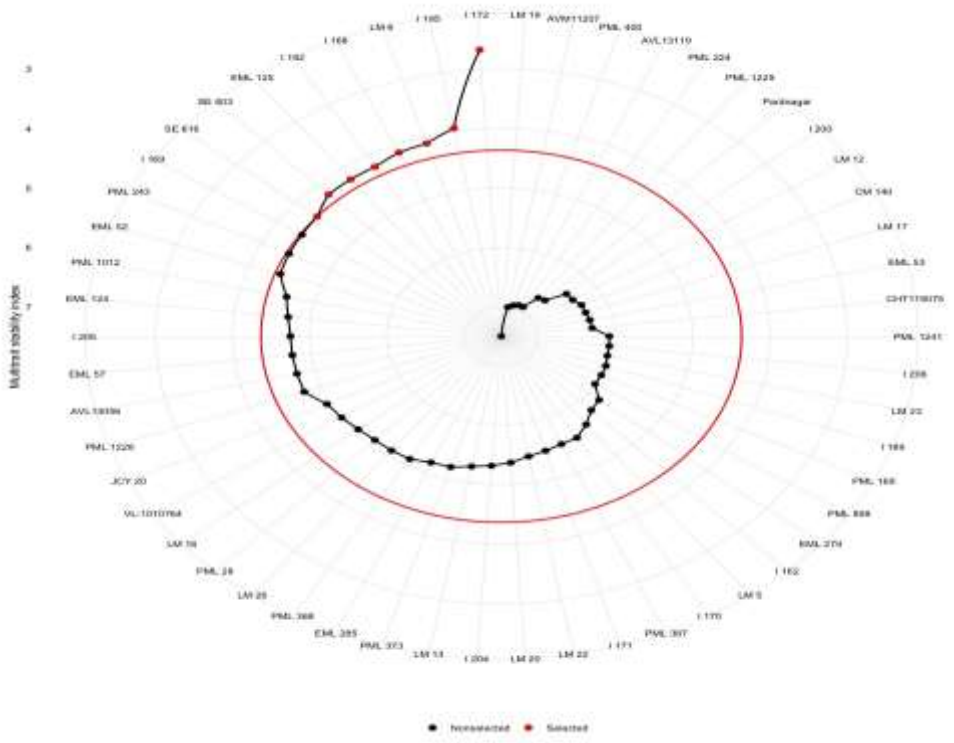


Fig. 4.14: Genotype ranking and selected genotypes among 54 maize inbreds for MTSI

4.5 Determination of waterlogging tolerance coefficient (WTC)

This parameter provides an important basis of comparison of genotypes by eliminating the differences among the lines under test. Since WTC overcome the inherent variance of characteristics among the test lines, this can be treated as an important index for measuring the level of susceptibility and tolerance. Nine seedling-traits from control and treatments were included in the study which includes, shoot dry weight (SDW), shoot fresh weight (SFW), shoot length (SL), root area (RA), root dry weight (RDW), root fresh weight (RFW), shoot length (SL), root area (RA) and shoot area (SA). An average value for each trait was calculated and WTC was determined defining the proportion change in mean trait value when subjected under stress. These values were presented in Table 4.22. Analysis of variance (ANOVA) showed significant difference among genotypes for all traits under study revealing at least two of the genotypes differ for the trait. To elucidate the result, Duncan's multiple range test (DMRT) was employed and thus obtained results presented as letters above the each value for each trait under study. The lines with same letters indicate no significant variation amongst while different letters indicate significant difference between the lines under study.

When WTC was calculated for SFW, the highest was recorded for EML 125 (1.19), for RFW the highest was for PML 373 (1.17). SDW showed highest value for I 192 (0.96), while RDW was recorded highest for LM 16 (0.9). For SL and RL the highest value were recorded for LM 16 (0.9) and I 172 (1.03) respectively. Shoot area (SA) recorded highest WTC for EML 125 with value 1.13. For RA, the highest WTC was recorded for PML 373 (1.56) and RV was highest for I 172 (1.05). Lines having values closer to one for all the traits considered provide confidence for selection of line against the stress considered. The results showed that lines EMl 125, EML 124, EML 53, EML 57, I 168, I 172, I 204, PML 373, PML 387. SE 503 and *Z. mays spp parviglumis* have their values nearer to or above one for all nine traits considered. Liu *et al* (2010) used WTC for screening of maize at seedling stage.

Data were collected from maize seedlings from the control plot and treatment plot, in which stress was provided for 9 days after plant attains V_2 stage and nine phenotypic data were observed.

WTC values followed by same letters across a column are non-significant while, different letters showed significance at P = 0.05 using Duncan's Multiple Range test.

Table 4.22: Screening of genotypes based on WTC value for 9 phenotypic characters along with the results from DMRT test showing genotypes with similar values represented by same letter

S. No.	Genotypes	WTC for nine phenotypic characters								
		SFW	RFW	SDW	RDW	SL	RL	SA	RA	RV
1	**EML 125**	1.19^{a}	0.9^{ij}	0.49^{pqrs}	0.56^{vwxy}	0.75^{ab}	$0.85^{defghijklmn}$	1.13^{a}	0.73^{jkl}	0.72^{nopqrs}
2	**EML 124**	0.73^{ijk}	1.04^{def}	0.49^{pqrs}	0.77^{fghijk}	0.81^{ab}	0.98^{abcd}	0.7^{ef}	1.21^{c}	0.74^{lmpqr}
3	**EML 285**	0.81^{gh}	0.91^{ij}	0.61^{cdef}	0.61^{stuv}	0.81^{ab}	$0.95^{abcdefgh}$	0.87^{c}	0.86^{h}	0.87^{efg}
4	**EML 279**	0.43^{y}	0.66^{r}	0.36^{z}	0.83^{bcde}	0.65^{ab}	$0.8^{jklmnopq}$	0.84^{cd}	0.53^{tuv}	0.76^{klmno}
5	**EML 52**	0.49^{uvw}	0.65^{r}	0.38^{yz}	0.81^{bcdef}	0.66^{ab}	0.77^{lmnopq}	0.39^{qr}	0.4^{yz}	0.87^{defg}
6	**EML 53**	1.06^{b}	0.89^{jk}	0.49^{opqrs}	0.54^{xyz}	0.75^{ab}	$0.92^{abcdefijk}$	0.31^{s}	1.09^{de}	0.6^{wxy}
7	**EML 57**	0.65^{lm}	0.95^{hi}	0.63^{cdef}	0.84^{bc}	0.81^{ab}	$0.85^{defijklmn}$	1.02^{b}	0.79^{ij}	1.01^{b}
8	**AVL13056**	0.93^{d}	0.89^{jkl}	0.47^{qrstu}	0.68^{mnopq}	0.79^{ab}	$0.92^{abcghij}$	0.53^{kl}	1^{fg}	0.77^{ijklmn}
9	**AVL13119**	0.85^{fg}	1.05^{de}	0.35^{zA}	0.61^{stuv}	0.73^{ab}	$0.86^{bchijklm}$	0.88^{c}	1.02^{efg}	0.7^{opqrst}
10	**AVM11207**	0.44^{xy}	0.46^{v}	0.56^{hijkl}	0.93^{a}	0.71^{ab}	0.76^{lmnopq}	0.64^{ghi}	0.47^{vw}	0.74^{lmpqr}
11	**CHT119075**	0.69^{kl}	0.76^{op}	0.46^{rstuv}	0.77^{fghij}	0.73^{ab}	$0.78^{klmnopq}$	0.48^{klmn}	0.7^{klmn}	0.69^{rstu}
12	**CM 140**	0.37^{z}	0.59^{s}	0.51^{nopq}	0.46^{AB}	0.67^{ab}	0.77^{lmnopq}	0.64^{ghi}	0.68^{lno}	0.82^{ghi}
13	**I 162**	0.56^{pqrst}	0.51^{u}	0.6^{fgh}	$0.69l^{mnpq}$	0.69^{ab}	0.75^{lmnopq}	0.46^{nop}	0.55^{rst}	0.63^{vw}
14	**I 168**	1.15^{a}	0.99^{fgh}	0.52^{lmnop}	0.91^{a}	0.83^{ab}	$0.92^{abcdehijk}$	0.46^{nop}	1.03^{efg}	0.7^{qrstu}
15	**I 169**	0.9^{de}	0.91^{ij}	0.69^{a}	0.73^{ijklm}	0.78^{ab}	$0.87^{bchijklm}$	0.88^{c}	0.56^{stu}	0.81^{hijk}
16	**I 170**	0.64^{mn}	1.05^{de}	0.54^{jklmn}	0.62^{rstuv}	0.76^{ab}	$0.86^{bchijklm}$	0.59^{j}	0.79^{ij}	0.8^{hijkl}
17	**I 171**	0.47^{vwxy}	0.79^{no}	0.56^{hijk}	0.78^{efghi}	0.77^{ab}	$0.82^{ijklopq}$	0.6^{ghij}	0.63^{nq}	0.51^{b}

S. No.	Genotypes	WTC for nine phenotypic characters								
		SFW	RFW	SDW	RDW	SL	RL	SA	RA	RV
18	**I 172**	1.05^{b}	1.17^{bc}	0.64^{bcde}	0.83^{bcd}	0.84^{ab}	1.03^{a}	0.44^{nopq}	0.77^{ijk}	1.05^{b}
19	**I 185**	0.84^{mn}	1.01^{efg}	0.95^{stuvw}	0.7l^{mnop}	0.84^{ab}	0.96^{abcdefg}	0.8^{d}	0.99^{g}	0.71^{opqrst}
20	**I 189**	0.53^{rstu}	0.55^{stu}	0.71 ^{hijk}	0.6^{tuv}	0.79^{ab}	0.69^{pq}	0.59^{hij}	0.65^{mnop}	0.89^{cdef}
21	**I 192**	0.6^{mnopq}	0.84^{lm}	0.96^{a}	0.97^{fghijk}	0.73^{ab}	0.81^{jklmnopq}	0.43^{opqr}	0.91^{h}	0.95^{c}
22	**I 200**	0.76^{ij}	0.58st	0.68^{ab}	0.84^{bc}	0.7^{ab}	0.72^{nopq}	0.52^{kl}	0.5^{uvw}	0.47^{a}
23	**I 204**	0.57^{opqrs}	1.15^{c}	0.5^{nopqr}	0.77^{fghij}	0.78^{ab}	0.94^{abcdefghi}	0.3st	1^{g}	0.82^{ghij}
24	**I 205**	0.56^{qrst}	0.66^{r}	0.44^{tuvwx}	0.83^{bcde}	0.67^{ab}	0.76^{lmnopq}	0.7^{ef}	0.86^{h}	0.92^{cd}
25	**I 206**	0.48^{uvwx}	0.59^{s}	0.49^{pqrs}	0.72^{jklmn}	0.69^{ab}	0.76^{lmnopq}	0.44^{nopq}	0.67^{lmno}	0.87^{efg}
26	**JCY 20**	0.92^{de}	0.87^{jkl}	0.48^{pqrst}	0.6^{tuv}	0.8^{ab}	0.95^{abcdefghi}	0.39^{qr}	0.98^{g}	0.75^{lmnop}
27	**LM 12**	0.56^{opqrst}	0.69^{qr}	0.56^{hijkl}	0.65^{pqrst}	0.73^{ab}	0.71^{opq}	0.72^{e}	0.52^{tuv}	0.74^{lmnopqrs}
28	**LM 13**	0.62^{mno}	0.69^{qr}	0.57^{ghij}	0.79^{cdefg}	0.68^{ab}	0.75^{lmnopq}	0.38^{r}	0.59^{pqrst}	0.69^{rstu}
29	**LM 16**	0.71^{jk}	0.72^{pq}	0.61^{def}	0.74^{hijkl}	0.9^{a}	0.89^{bcdehijkl}	0.54^{k}	0.57^{qrstu}	0.88^{def}
30	**LM 17**	0.99^{c}	1.41^{a}	0.59^{fghi}	0.59^{uvwx}	0.85^{ab}	0.96^{abcdef}	0.6^{hij}	1.26^{bc}	0.7^{pqrst}
31	**LM 19**	0.29^{AB}	0.32^{x}	0.42^{vwx}	0.75^{ghijk}	0.71^{ab}	0.69^{q}	0.26^{tu}	0.36^{z}	0.66^{tuv}
32	**LM 20**	0.45^{wxy}	0.73^{pq}	0.5^{nopqr}	0.6^{tuv}	0.69^{ab}	0.83^{ghijklmno}	0.49^{klmn}	0.62^{opqr}	0.52^{zA}
33	**LM 22**	0.57^{opqr}	0.81^{mn}	0.45^{stuvwx}	0.78^{efghi}	0.73^{ab}	0.85^{defhklmn}	0.51^{klm}	0.68^{lmno}	0.78^{ijklm}
34	**LM 23**	0.6^{mnopq}	0.58st	0.57^{ghij}	0.44^{B}	0.73^{ab}	0.78^{lmnopq}	0.41^{pqr}	0.61^{opqrs}	0.57^{xyz}
35	**LM 26**	0.61^{mno}	0.65^{r}	0.52^{klmnop}	0.6^{tuv}	0.71^{ab}	0.82^{hijklmnop}	0.53^{kl}	0.71^{klm}	0.61^{vwx}
36	**LM 5**	0.76^{hi}	0.68^{qr}	0.65^{bcd}	0.52^{yz}	0.74^{ab}	0.83^{fghijlmno}	0.48l^{mno}	0.54^{stuv}	0.9^{cde}
37	**LM 6**	0.62^{mno}	1.01^{efg}	0.65^{bc}	0.78^{defgh}	0.82^{ab}	0.86^{cdefgjklm}	0.65^{fg}	0.97^{g}	0.77^{jklmn}

S. No.	Genotypes	WTC for nine phenotypic characters								
		SFW	RFW	SDW	RDW	SL	RL	SA	RA	RV
38	**PML 1012**	0.6^{nopq}	0.52^{u}	0.49^{opqrs}	0.52^{yz}	0.73^{ab}	$0.84^{efghijmno}$	0.59^{hij}	0.73^{jklm}	0.7^{pqrstu}
39	**PML 1228**	0.76^{ij}	0.96^{h}	0.52^{klmnop}	0.66^{pqrs}	0.74^{ab}	$0.87^{bcghijklm}$	0.59^{ij}	0.72^{jklm}	0.55^{yzA}
40	**PML 1229**	0.51^{tuv}	0.38^{w}	0.68^{ab}	0.5^{zA}	0.72^{ab}	0.74^{mnopq}	0.4^{qr}	0.53^{stuv}	0.53^{zA}
41	**PML 1241**	0.26^{B}	0.5^{uv}	0.54^{jklmn}	0.66^{opqr}	0.67^{ab}	0.76^{lmnopq}	0.3^{st}	0.5^{uvw}	0.65^{uvw}
42	**PML 169**	0.6^{nopq}	0.69^{qr}	0.51^{mnopq}	0.59^{uvw}	0.77^{ab}	$0.83^{hijklmnop}$	0.6^{ghij}	0.63^{nopq}	0.55^{yzA}
43	**PML 224**	0.52^{rstu}	0.4^{w}	0.53^{jklmno}	0.6^{tuv}	0.66^{ab}	0.72^{nopq}	0.25^{u}	0.42^{xyz}	0.51^{A}
44	**PML 243**	0.75^{ij}	0.75^{op}	0.41^{wxy}	0.76^{fghijk}	0.8^{ab}	$0.89^{bcdghijkl}$	0.8^{d}	0.9^{h}	0.94^{c}
45	**PML 28**	0.85^{fg}	0.66^{r}	0.6^{efg}	0.66^{opqr}	0.78^{ab}	$0.88^{bcdefijkl}$	0.52^{klm}	0.74^{jkl}	0.75^{lmnopq}
46	**PML 368**	1.08^{b}	1.07^{d}	0.43^{uvwx}	0.67^{nopq}	0.76^{ab}	$0.94^{abcdghij}$	0.85^{c}	0.89^{h}	0.68^{stu}
47	**PML 373**	1.15^{a}	1.17^{bc}	0.32^{A}	0.85^{b}	0.72^{ab}	0.99^{abc}	1.03^{b}	1.56^{a}	0.78^{ijklm}
48	**PML 387**	1.05^{b}	1.21^{b}	0.62^{cdef}	0.71^{klmno}	0.87^{ab}	0.97^{abcde}	1.05^{b}	0.83^{hi}	$0.74^{lmnopqr}$
49	**PML 400**	0.52^{stuv}	0.52^{u}	0.63^{cdef}	0.71^{klmno}	0.76^{ab}	0.74^{mnopq}	0.48^{klmno}	0.56^{qrstu}	$0.74^{mnopqrs}$
50	**PML 898**	0.33^{zA}	0.53^{tu}	0.36^{z}	0.55^{wxyz}	0.6^{b}	0.77^{lmnopq}	0.28^{stu}	0.44^{wxy}	$0.73^{mnopqrs}$
51	**SE 503**	0.87^{ef}	0.97^{gh}	0.55^{ijklm}	0.73^{ijklm}	0.82^{ab}	1.02^{a}	0.85^{c}	1.31^{b}	0.87^{efg}
52	**SE 616**	0.61^{mnop}	0.85^{klm}	0.41^{xy}	0.62^{rstuv}	0.65^{ab}	$0.89^{bcdefghijkl}$	0.65^{fgh}	0.6^{pqrst}	1.04^{b}
53	***Z. mays spp parviglumis***	1.05^{b}	1.14^{c}	0.48^{pqrst}	0.7^{lmnop}	0.84^{ab}	1^{ab}	1.1^{a}	1.07^{def}	0.54^{yzA}
54	**VL-1010764**	0.84^{fg}	0.97^{gh}	0.56^{ghij}	0.64^{qrstu}	0.81^{ab}	$0.86^{cdefghijklm}$	0.61^{ghij}	1.1^{d}	0.84^{fgh}

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), shoot area (SA), root (RA) and root volume (RV)

WTC values followed by same letters across a column are non-significant while, different letters showed significance at P=0.05 using Duncan's Multiple Range test.

4.6 Anthesis Silking Interval (ASI)

ASI is an important parameter for defining the pollination and grain setting in maize. Maize being protandrous crop, anthesis occurs earlier than silking and the pollination efficacy is determined by how long the pollens remain viable and how receptive the silk remains. Normally the ASI interval for maize remains around 2-3 days under optimal growth condition and this synchronous flower habit ensures the good pollination in maize, but under the stress condition, mostly abiotic stress, this period widens and the synchronization is lost. Though precise mechanism behind this discrepancy has not been known, lengthen ASI is mostly attributed to prolonged period for silking as silking is assumed to be severely affected under stress condition. Under waterlogging, we observed the similar modification in the ASI period and genotypes exhibited the variation based on the tolerance and susceptibility. In the selected lines, 43 lines showed both anthesis and silking while rest lacked either silking or both. Some tolerant lines were able to overcome the effects of waterlogging and maintain the normal ASI of 2-4 days, while the susceptible lines showed the broaden ASI with some of them exhibiting as long as 8 days. In general, the result from the ASI study orchestrated that with the increase in the duration of the waterlogging, the normal flowering in maize went on worsening and malformation of the cobs became more prominent. At 3 days of seedling stage waterlogging, plants did not show any change in ASI but as the duration was changed to 6 and 9 days, variation in the ASI became more significant and results showed among all treatments, 9 days of waterlogging at the seedling stage to be severely affecting the ASI. Normal ASI (≤ 4 days) even at 9 days of waterlogging were shown by EML 124, EML 125, I 168, I 169A, I 170, I 172, I 192, JCY 20, LM 16, LM 17, LM 26, PML 1228, PML 243, PML 387, SE 503 and SE 616. Prolonged ASI (≥ 6) were seen in AVL13119, EML 279, EML 52, I 162, I 171, I 185, I 188, I 200, I 204, I 205, I 206, LM 12, LM 13, LM 20, LM 22, LM 23, LM 5, LM 6, PML 1012, PML 1229, PML 1241, PML 169, PML 368 and PML 400. The experimental findings were similar to one reported by Lone and Warsi (2009) where they presented that under excess moisture stress the ASI increased for over 8 days. A similar study by Tripathi *et al* (2003) showed that both silking and tasselling were delayed with increased ASI under waterlogged condition.

Genotypes with recorded ASI across different duration of waterlogging is presented in Table 4.23.

Table 4.23: Anthesis-Silking Interval (ASI) of lines at different duration of waterlogging

Genotypes	9 days of waterlogging			6 days of waterlogging			3 days of waterlogging			Control		
	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI
AVL13056	66	71	5	63	68	5	62	65	3	61	64	3
AVL13119	67	73	6	65	70	5	64	67	3	63	65	2
CM 140	64	69	5	63	67	4	58	61	3	57	60	3
EML 124	70	74	4	69	73	4	67	69	2	64	65	1
EML 125	66	70	4	58	61	3	58	61	3	56	58	2
EML 279	77	84	7	65	71	6	57	61	4	55	58	3
EML 52	76	83	7	68	73	5	60	64	4	60	63	3
EML 53	70	75	5	66	71	5	64	67	3	63	65	2
I 162	74	81	7	68	73	5	61	65	4	61	64	3
I 168	65	69	4	63	67	4	62	65	3	61	63	2
I 169A	66	69	3	65	68	3	62	65	3	62	64	2
I 170	66	70	4	65	68	3	62	65	3	61	64	3
I 171	69	76	7	68	75	7	58	61	3	56	59	3
I 172	72	76	4	68	71	3	61	64	3	58	61	3
I 185	76	83	7	72	76	4	66	70	4	66	69	3
I 188	66	74	8	63	68	5	60	64	4	60	64	4
I 192	66	70	4	65	68	3	60	63	3	59	61	2
I 200	70	77	7	62	68	6	60	64	4	58	61	3
I 204	70	78	8	68	74	6	65	69	4	63	66	3
I 205	69	77	8	67	73	6	65	69	4	63	67	4
I 206	66	74	8	63	69	6	62	65	3	62	65	3
JCY 20	73	77	4	62	66	4	59	62	3	57	59	2

Genotypes	9 days of waterlogging			6 days of waterlogging			3 days of waterlogging			Control		
	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI	Days to anthesis	Days to silking	ASI
LM 12	75	83	8	68	74	6	65	68	3	64	67	3
LM 13	82	90	8	79	85	6	64	68	4	57	61	4
LM 16	70	73	3	68	71	3	66	68	2	66	68	2
LM 17	60	64	4	59	63	4	58	62	4	57	61	4
LM 20	69	75	6	68	73	5	66	70	4	65	69	4
LM 22	76	82	6	75	80	5	65	68	3	63	66	3
LM 23	74	81	7	69	74	5	67	71	4	67	71	4
LM 26	79	83	4	71	75	4	69	72	3	66	69	3
LM 5	76	83	7	75	80	5	62	67	5	61	64	3
LM 6	68	75	7	62	68	6	59	63	4	58	62	4
PML 1012	69	76	7	67	72	5	62	65	3	61	63	2
PML 1228	65	69	4	64	67	3	64	67	3	63	65	2
PML 1229	79	87	8	76	81	5	67	71	4	66	70	4
PML 1241	72	79	7	69	74	5	68	72	4	67	71	4
PML 169	65	73	8	59	67	8	59	63	4	58	61	3
PML 243	76	80	4	75	78	3	69	72	3	68	70	2
PML 368	73	79	6	60	65	5	59	62	3	58	61	3
PML 387	73	76	3	63	66	3	60	62	2	60	62	2
PML 400	73	81	8	65	71	6	57	62	5	56	60	4
SE 503	63	66	3	62	65	3	57	59	2	57	59	2
SE 616	75	79	4	68	72	4	66	69	3	66	69	3

4.7 Aerenchyma formation:

To have a deeper understanding of the morphological changes that undergoes in root tissues when subjected to waterlogging stress, a thin cross section of the root at varying length was made and viewed under light microscope and image captured using camera attached to the computer. Plants subjected to waterlogging for 9 days were selected based on the phenotypic observation and plants showing extreme phenotypes; two tolerant, two susceptible and one *Z. mays spp parviglumis* were uprooted carefully, washed off the adhered dirt particles and kept in beaker full of water till section was made. Cross section was made using a sharp blade and acetocarmine dye was used to stain the slide.

All of the selected lines showed the aerenchyma formation when the section was cut at distance greater than 100 mm from the root tip. But the area of aerenchyma differ among them. Difference was more significant at 10 mm and 50 mm. To have a more precise estimation, the percentage of aerenchyma formed in the cross section was calculated by dividing the area of aerenchyma with the area of the root cross section. Area covered by the aerenchyma was measured by using ImageJ 1.53a software. List of selected genotypes, their tolerance level based on visual characteristics and the distance from the root tip from where the cross section has been made along with their aerenchyma percentage is presented in Table 4.24. Based on the aerenchyma study it became quite evident that this hollow air chamber passing throughout the root plays a significant role in conferring tolerance against waterlogging and the percentage aerenchyma formation in tolerant lines showed significant variation compared to the susceptible lines.

Results from the study showed that the tolerant lines (I 172 and PML 243) had significantly higher percentage aerenchyma at almost all three different length behind the root tip. One important observation obtained from the cross-section observation was the tolerant lines initiated the aerenchyma formation even at the tip (10 mm) of the freshly formed roots while it was lacking for susceptible lines. Highest aerenchyma formation was observed for *Z. mays spp parviglumis* at 50mm behind the root tip (31.17%) and for PML 243 at 110mm behind the root tip (19.88%). The susceptible lines too have aerenchyma formation at 50mm and 150mm but was completely absent at 10mm behind the root tip. Findings from the current study showed the similar result as demonstrated by Gong *et al* (2019) where it was presented that the lines with higher percentage aerenchyma formation had greater tolerance against waterlogging.

Aerenchyma has well known role in diffusion of oxygen from the upper exposed plant parts to the lower submerged portion for root respiration and the results from the study showing higher percentage aerenchyma formation for tolerant lines further suggested their similar role in conferring tolerance against waterlogging. Variation in percentage aerenchyma

formation across tolerant and susceptible lines in response to waterlogging can be observed in Plate 4.4.

Table 4.24: Percentage aerenchyma formation at different length behind root tip as measured by ImageJ software package

S. No.	Lines	Genotype name	Cross section taken behind root tip	Percentage aerenchyma
1	Tolerant	I 172	110 mm	11.66%
			50 mm	5.329%
			10 mm	11.39%
		PML 243	110 mm	19.88%
			50 mm	16.07%
			10 mm	8.08%
2	Susceptible	I 183	150 mm	7.86%
			50 mm	5.43%
			10 mm	0.00%
		LM 21	150 mm	13.38%
			50 mm	12.67%
			10 mm	0.00%
3	Tolerant	*Z. mays ssp. Parviglumis*	130 mm	17.05%
			50 mm	31.17%
			10 mm	0.00%

4.8 Barrier to Radial Oxygen Loss (ROL

To understand the ability of plant to form barrier against the radial oxygen loss to avoid the leakage of oxygen from the root before reaching the root tips, an experiment was conducted to visually observe the surface from which the oxygen was leaching out. 48 lines at 9 days of continuous waterlogging used for the experiment were subjected to test to detect the barrier formation. Blue staining from the oxidation of methylene blue indicates the loss of oxygen. Proportion of blue staining observed in the root segment is measured using ImageJ 1.53a software and recorded data is presented in percentage barrier formed and illustrated in Table 4.25.

Visual observation (Plate 4.5) for barrier formation against ROL showed that the tolerant lines had significant amount of barrier formation compared to susceptible lines. This barrier prevents the leakage of oxygen and ensures delivery of oxygen to the root tip where it is utilized for root respiration. The role of barrier to ROL has been extensively studied and reported by several researchers (Ejiri and Shiono 2019; Ejiri *et al* 2021). Genotypes exhibiting difference in percentage barrier formation is presented in Plate 4.6. Genotypes with increasing order of percentage barrier formation is shown.

Table 4.25: Percentage barrier formation against radial oxygen loss across different genotypes

Line No.	Genotypes	% barrier	Line No.	Genotypes	% barrier
154	**AVL13056**	72.15	46	**LM 12**	0
130	**AVL13119**	92.39	47	**LM 13**	20
14	**CM 140**	13.68	49	**LM 16**	90.32
124	**EML 124**	85.84	50	**LM 17**	77.06
99	**EML 125**	88.01	52	**LM 20**	23.31
123	**EML 279**	25.02	53	**LM 22**	39.63
3	**EML 285**	79.19	54	**LM 23**	46.03
84	**EML 52**	38.28	56	**LM 26**	81.98
89	**EML 53**	89.52	57	**LM 5**	21.35
12	**EML 57**	82.58	58	**LM 6**	82.29
91	**I 162**	37.47	59	**PML 1012**	29.1
92	**I 168**	92.82	60	**PML 1228**	86.51
28	**I 170**	36.87	140	**PML 1241**	30.58
29	**I 171**	0	132	**PML 169**	54.94
30	**I 172**	90.85	148	**PML 368**	71.1
133	**I 185**	94.19	152	**PML 373**	80.25
90	**I 189**	0	65	**PML 387**	91
37	**I 192**	82.77	87	**SE 503**	84.09
40	**I 204**	89.71	138	**PML 28**	92.61
41	**I 205**	0	82	**SE 616**	19.58
42	**I 206**	0	86	**VL-1010764**	84.75
145	**JCY 20**	80.25	161	***Z. mays spp parmigulmis***	91.52

4.9 DUS characterization

With an aim to define distinctness, uniformity and stability (DUS) of the 80 maize inbred lines procured from the department of Plant Breeding and Genetics, PAU, an experiment was carried out in maize trial experimental area at PAU. The inbred lines were sown in randomized block design (RBD) in three replications in the year 2020. Several morphological data were recorded by visual observations, and others from the measurement of those characters. Data collected during the experiment were analysed and results obtained were presented under different sections.

4.9.1 Morphological characterization based on DUS descriptors for Maize:

A total of 28 morphological characteristics were taken into account to distinguish 80 maize inbred lines which included 20 visually assessed characteristics and 8 measurable characteristics.

4.9.1.1 Measurable DUS characteristics

Eight measurable DUS characteristics were employed for collection of traits. Traits such as Plant height, Width of leaf blade, Anthesis period, length of tassel, silking period, cob length, cob diameter and grain weight were taken as measurable DUS characters. Vernier Calliper was employed for taking cob length and cob diameter, while a long (1 meter) scale for plant height and leaf blade widt. 1000 grain weight was measured using standard weighing balance. Data from measurable DUS characteristics are presented in Table 4.26. Detailed data recorded for both visually assessed and measurable characteristics is presented in Table 4.27.

Data showed diversity for measurable DUS characteristics. Data for Time of silk emergence and Time for anthesis showed that none of the inbred lines showed very early (<45 days) state of silk emergence and anthesis. Few row number (≤8) for the trait: Number of rows of grains was not recorded for any of the lines under study. For kernel size (1000 kernel weight), none of the lines fell on very small (<100g) category. Most of them have either small (100-200g) or medium (200-300g) test weight. Five lines; PML 243, PML 1231, CML 451, PML 387 and PML 1230 have the test weight greater than 300g. Least test weight was recorded for SE 601 (117.01g). Different visually assessed and measurable traits considered for the experiment were photographed and presented in Plate 4.7.

4.9.1.2 Visually assessed DUS characteristics

Data showed significant variation for almost all the traits considered except for trait: Type of the grain. Since all the maize lines chosen for the experiment were field corn, no entry was recorded for popcorn, waxy corn, opaque corn and sweet corn. Yellow type of colour of grain was recorded for PML 1012, while variegated was recorded for SE 613. I 179 and I 202 have red type of grain colour. None of the grain have white and white with cap state of grain colour. Accordingly, almost every lines showed white colouration of glumes of cob with exception for only two lines LM 21 and SE 607, which showed light purple colouration. None of the lines showed Dark purple colouration. A single line SE 601 showed shrunken type of kernel shape while the most from the rest were round or toothed and few being indented. None of the line showed pointed type of kernel shape. Data with different level of visual DUS characters were code labelled and presented in Table 4.27. Different visually assessed traits considered for the experiment were photographed and presented below.

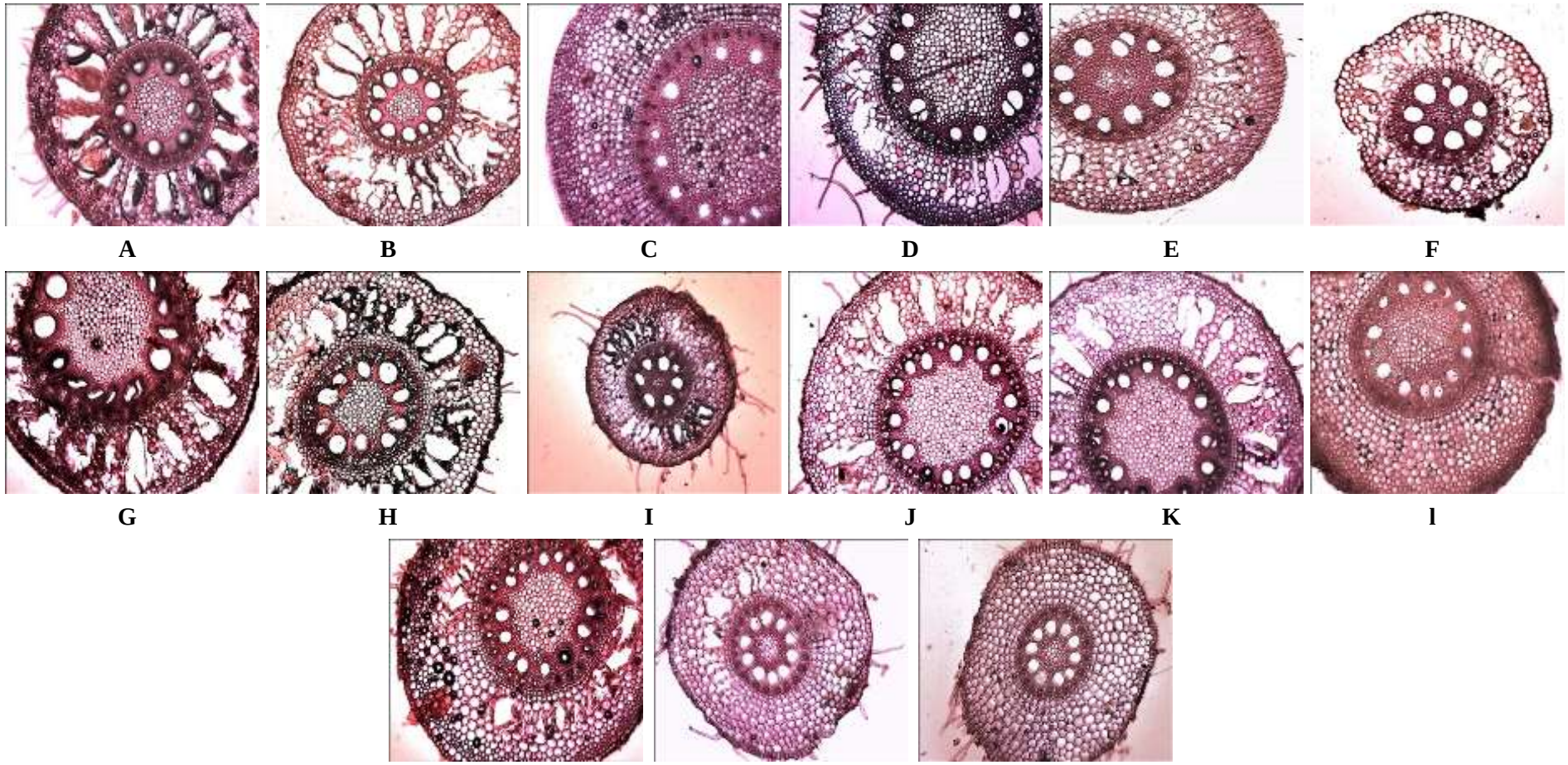


Plate 4.4: A, B, C- Root cross section of *Z. mays spp parviglumis* at 130 mm, 50 mm and 10 mm respectively from the root tip, D, E, F- Root cross section of I 172 at 110 mm, 50 mm and 10 mm respectively from the root tip, G, H, I- Root cross section of PML 243 at 110 mm, 50 mm and 10 mm respectively from the root tip, J, K, L- Root cross section of I 183 at 110 mm, 50 mm and 10 mm respectively from the root tip, M,N,O- Root cross section of LM 21 at 110 mm, 50 mm and 10 mm respectively from the root tip

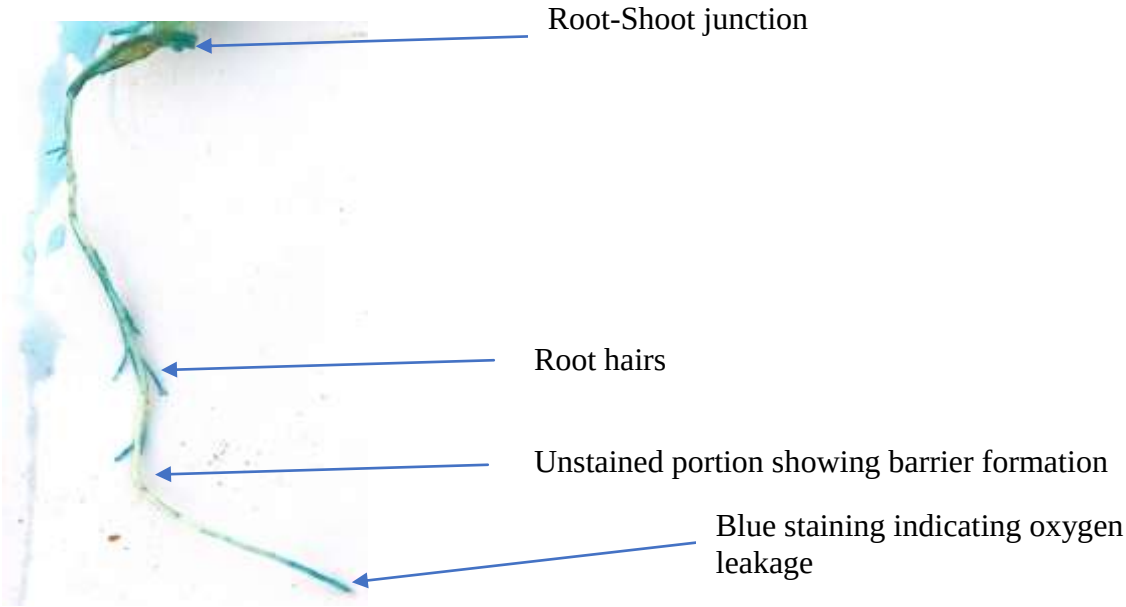
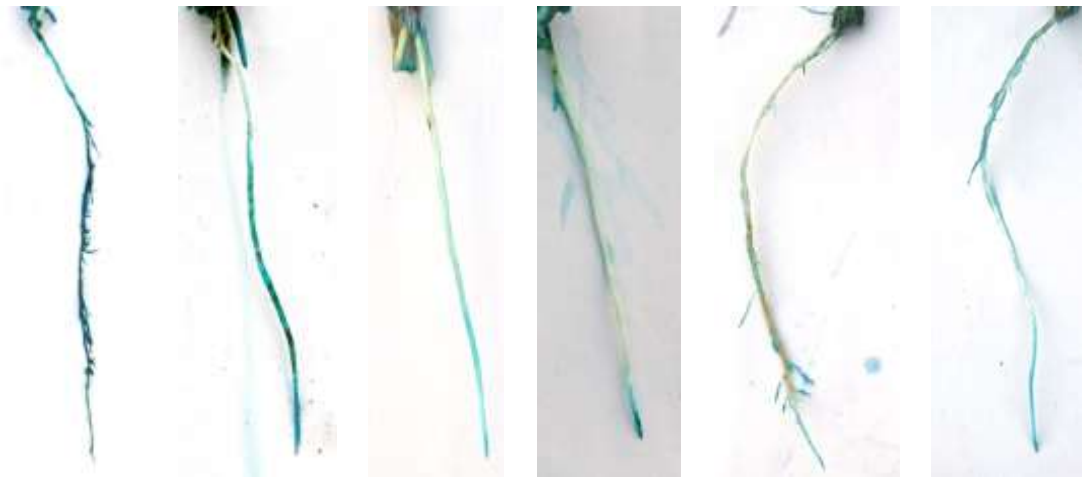


Plate 4.5: Visual observation of root showing barrier to ROL formation



I 171 (0%)	LM 13 (20%)	LM 23 (46.03%)	EML 285 (79.19%)	PML 28 (92.61%)	*Z. mays spp parviglumis (91.52%)*

Plate 4.6: Visual observation of barrier formation against ROL across different inbred lines

Table 4.26: Genotypes with data recorded for several measurable DUS characters.

Genotypes	Cob length (cm)	Cob diameter (cm)	Tassel length (cm)	Plant height (cm)	Siliking (days)	leaf blade width (cm)	Anthesis (days)	Kernel wt (1000grain) (g)
LM 6	12.85	3.26	29	126.33	57	6.5	55	188.7
LM 21	12.89	4	34.67	132.33	55	10.47	53	295.51
LM 13	13.16	3.53	29.33	150.33	55	8.23	53	274.92
LM 24	12.35	2.83	19.67	105.33	58	6.9	56	278.97
PML 1224	10.6	3.85	18.33	143.33	55	8.53	54	222.99
PML 387	12.45	3.76	21.67	172.67	55	7.1	54	336.16
PML 503	11.69	3.63	22.33	110.67	57	6.03	56	222.32
LM 14	14.04	3.87	30.67	159.33	56	7.83	54	205.67
PML 920	11.12	3.42	27.67	99.33	56	7.03	55	281.53
PML 368	11.4	3.2	17.67	120.33	58	6.77	56	279.48
PML 1229	9.43	3	33.33	105	54	6.73	52	208.97
PML 333	10.34	4.12	36.67	104.33	56	6.9	54	150.36
PML 1225	12.32	3.31	29.33	119.33	56	6.83	54	222.54
PML 41	6.91	3.79	17	109.67	62	5.8	60	248.5
PML 243	10.43	4.08	29	151.67	54	8.3	53	312.68
PML 1012	12.17	2.97	35	127	56	6.13	54	204.84
PML 1231	11.97	3.85	36.33	100.33	54	7.13	53	339.78
PML 892	11.02	3.92	36.33	109	59	6.43	57	268.52
CML 451	12.09	3.4	30.33	112	55	9.8	53	302.53
PML 1230	10.48	3.32	25.67	143.33	55	8.2	53	340.62

Genotypes	Cob length (cm)	Cob diameter (cm)	Tassel length (cm)	Plant height (cm)	Siliking (days)	leaf blade width (cm)	Anthesis (days)	Kernel wt (1000grain) (g)
CM 140	10.87	3.61	27	128.67	51	6.9	49	174.31
l 105A	9.63	3.99	20	111.33	52	7.53	50	160.8
l 108	8.53	3.33	16.33	108.67	50	6.4	48	140.76
l 121	10.57	3.22	22.33	101.67	52	7.13	50	185.18
I 132	9.67	3.74	30.67	104.67	52	6.3	50	205.98
I 161A	10.87	3.62	27	128.33	56	8.63	54	164.73
I 164	11.88	3.02	30.67	109.33	53	6.8	51	160.22
I 165	12.44	3.27	19.67	110.67	53	6.73	53	213.61
I 171	15.3	2.69	35.67	136.67	53	6.83	51	126.91
I 172	18.4	2.76	28	133.67	52	5.83	50	161.66
I 173	17.04	2.98	31	156.67	54	8.83	53	171.68
I 177	9.89	3.14	22	119	54	6.5	53	162.36
I 179	9.04	3.47	26.67	95.33	52	6.37	50	226.59
I 182	10.57	3.59	30.33	122	53	5.67	51	172.13
I 187	10.91	3.34	25.33	104.67	55	5.8	53	178.08
I 230	12.26	2.91	25	138.67	53	7.1	51	121.13
I 231	9.24	3.32	23	93.33	54	6.53	52	191.47
I 190	8.6	4.07	20.33	110.67	52	7.37	51	174.48
EL 1	12.52	2.96	23.33	137.33	53	7.27	52	152.19
EL 2	11.58	3.47	34.33	130.33	52	5.9	51	211.37
I 200	10.06	3.43	34	152.67	53	6.13	52	130.72

Genotypes	Cob length (cm)	Cob diameter (cm)	Tassel length (cm)	Plant height (cm)	Siliking (days)	leaf blade width (cm)	Anthesis (days)	Kernel wt (1000grain) (g)
I 202	9.12	4.01	26.33	135.33	52	7.33	50	210.32
I 205	10.93	3.84	20.67	125.33	54	7.07	54	162.82
I 206	6.74	3.35	18.33	96	51	6.73	50	120.43
I 169	13.67	3.7	30	160	52	8.4	51	235.07
I 188	10.83	3.7	28.33	130.33	54	7.13	53	184.27
CM 139	12.61	3.25	31.67	103	50	6.03	48	210.11
SE 533A	10.88	3.21	24	99	52	7.37	50	168.09
CML 32	9.74	3.64	29.33	122	52	6.4	51	158.85
SE 544	11.51	3.46	24.67	110.67	54	6.33	52	138.26
SE 547	11.24	3.16	38	114	51	5.9	49	162.4
SE 549	9.07	3.26	35.67	106.67	54	7.07	53	167.44
SE 557	9.25	3.01	22.67	133.67	53	5.67	50	166.47
LM 22	10.39	3.29	32.67	144.33	56	7.03	54	164.86
LM 23	11.4	3.66	33.33	136.67	54	6.77	52	149.21
SE 563	13.61	2.59	23	108.67	51	8.57	49	140.41
SE 567	13.11	3.59	33.33	138.67	56	5.77	53	170.75
SE 571	13.44	3.24	30.33	102.67	54	8.63	53	188.28
EML 57	11.9	3.54	17.33	113.33	55	7.4	54	145.24
SE 601	12.85	4.08	32.33	140.67	55	8.4	54	117.01
SE 603	13.11	2.11	33	130.33	55	7.5	53	170.87
SE 604	14.68	2.95	31.67	135.33	52	7.3	50	223.8

Genotypes	Cob length (cm)	Cob diameter (cm)	Tassel length (cm)	Plant height (cm)	Siliking (days)	leaf blade width (cm)	Anthesis (days)	Kernel wt (1000grain) (g)
SE 607	10.36	3.14	17	109	52	9.57	50	134.25
SE 610	14.21	4.06	28	162.67	56	10.57	55	242.18
SE 613	10.87	3.18	18.67	147	52	9.2	50	185.37
SE 616	12.64	3.83	35.33	160	55	8.67	52	235
I 183	8.39	3.42	19.67	102	55	6.3	52	226.84
SE 503	12.17	3.33	37	131	56	7.3	55	171.78
EML 282	10.32	3.53	29.33	140	53	6.3	53	235.23
EML 132	12.51	3.16	32.67	140	53	7.03	51	242.37
LM16 Zp1	12.64	2.99	37	165	51	8.33	50	235.22
SE 616	13.58	3.83	34	172.33	50	9.3	48	237.83
EML 122	13.9	3.69	23	144.67	51	8.43	48	215.68
EML 240	8.33	3.08	33.67	106	55	6.17	53	239.34
EML 126	12.41	3.82	22.67	107	55	7.5	53	242
EML 285	11.48	3.32	31.67	146.33	57	7.03	56	246.52
EML 272	13.14	3.38	25	94.67	55	6.6	54	190.33
EML 283	12.16	3.46	23.33	98.33	55	5.7	54	261.15
LM 16	11.23	3.39	24	144.33	52	6.07	50	190.44
LM 20	13.9	3.15	31.67	108.33	54	10.47	52	224

Table 4.27: Morphological characterization according to DUS parameters in 80 maize inbred lines

Genotype	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
LM 6	1	1	9	7	9	9	9	7	1	5	5	1	1	5	5	5	3	5	3	3	5	1	4	1	1	1	2	3
LM 21	1	9	9	5	9	9	1	3	9	5	5	9	1	7	3	5	7	5	5	3	7	1	4	2	1	1	4	5
LM 13	1	1	1	5	9	9	1	3	1	1	5	9	1	5	5	7	5	5	3	2	7	1	4	1	1	1	4	5
LM 24	1	1	9	7	9	9	9	3	9	5	7	9	1	3	5	3	3	5	3	1	5	1	5	1	1	3	4	5
PML 1224	1	1	9	5	9	9	1	7	1	1	5	9	1	3	3	5	5	5	3	3	7	1	5	1	1	1	3	5
PML 387	1	1	9	5	9	9	1	3	1	5	5	9	1	5	7	7	3	5	3	3	5	1	5	1	1	1	4	7
PML 503	1	1	9	7	9	9	9	7	1	1	5	1	1	5	5	3	3	5	3	1	7	1	5	1	1	1	4	5
LM 14	1	9	9	5	1	9	1	7	9	5	5	9	1	7	7	7	5	5	3	1	7	1	5	1	1	3	2	5
PML 920	1	9	9	7	9	9	9	7	1	1	5	9	1	5	5	3	3	5	3	3	5	1	4	1	1	3	2	5
PML 368	1	1	9	7	9	9	1	7	1	5	7	9	1	3	7	5	3	5	3	3	5	1	5	1	1	1	2	5
PML 1229	1	1	1	5	1	1	1	7	1	1	5	9	1	7	5	3	3	3	3	3	5	1	5	1	1	3	3	5
PML 333	1	9	9	5	1	1	1	3	1	5	5	1	1	7	5	3	3	5	5	1	5	1	5	1	1	3	4	3
PML 1225	1	9	1	5	1	1	1	3	1	5	5	1	1	5	5	3	3	5	3	3	5	1	5	1	1	3	2	5
PML 41	1	1	9	7	1	1	9	3	1	1	7	9	1	3	5	3	3	3	3	3	7	1	5	1	1	1	2	5
PML 243	1	1	9	5	9	9	1	7	1	1	5	1	1	5	5	5	5	5	5	3	7	2	4	1	1	1	3	7
PML 1012	1	1	9	5	1	9	1	7	1	5	5	9	1	7	5	5	3	5	3	3	5	1	3	1	1	1	3	5
PML 1231	1	1	9	5	9	1	9	7	1	1	5	9	1	7	5	3	3	5	3	1	5	3	4	1	1	1	3	7
PML 892	1	1	1	7	9	9	1	7	1	1	7	9	9	7	5	3	3	5	3	3	7	1	5	1	1	1	4	5
CML 451	1	1	9	5	1	9	1	7	1	1	5	9	1	5	3	3	7	5	3	3	7	1	4	1	1	3	4	7
PML 1230	1	1	9	5	1	9	1	7	1	5	5	9	9	5	7	5	5	5	3	1	5	1	5	1	1	3	4	7
CM 140	1	1	1	3	1	1	1	3	1	1	3	1	1	5	3	5	3	5	3	3	5	1	5	1	1	2	2	3

Genotype	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
l 105A	9	9	1	5	1	1	1	7	9	5	3	1	1	5	5	3	3	3	3	1	7	1	5	1	1	3	2	3
l 108	9	9	1	3	1	1	1	3	9	1	3	1	1	3	5	3	3	3	3	3	5	1	5	1	1	1	2	3
l 121	1	1	1	5	1	1	1	7	1	1	3	9	1	5	3	3	3	5	3	3	5	1	5	1	1	3	2	3
I 132	9	1	9	5	1	1	1	7	1	5	3	1	1	5	5	3	3	3	3	1	7	1	5	1	1	1	2	5
I 161A	1	1	9	5	1	1	1	7	1	5	5	1	1	5	5	5	5	5	3	1	5	1	5	1	1	1	2	3
I 164	1	9	9	5	1	1	1	7	1	5	5	1	1	7	3	3	3	5	3	3	7	1	5	1	1	1	4	3
I 165	9	1	1	5	1	1	1	7	1	1	5	1	1	5	3	3	3	5	3	3	5	1	5	1	1	1	4	5
I 171	9	9	1	5	1	1	1	7	1	5	5	1	1	7	7	5	3	7	3	3	5	1	5	1	1	2	2	3
I 172	9	9	1	5	1	1	1	7	1	5	3	1	1	5	7	5	3	7	3	3	5	1	5	1	1	2	2	3
I 173	9	9	1	5	1	1	1	7	1	5	5	1	1	7	5	7	5	7	3	3	5	1	5	1	1	2	2	3
I 177	1	1	1	5	1	9	1	7	1	5	5	1	1	5	5	3	3	3	3	1	5	1	5	1	1	2	2	3
I 179	1	9	1	5	1	1	1	7	1	1	3	1	1	5	5	3	3	3	3	3	7	1	6	1	1	2	2	5
I 182	1	9	1	5	1	1	1	7	1	5	5	1	1	7	3	5	3	5	3	3	7	1	5	1	1	1	3	3
I 187	9	1	1	5	1	1	9	7	9	1	5	1	1	5	3	3	3	5	3	1	7	1	5	1	1	1	2	3
I 230	9	9	1	5	1	1	1	7	1	5	5	1	1	5	5	5	3	5	3	3	5	1	5	1	1	1	2	3
I 231	9	9	9	5	1	1	1	7	1	1	5	1	1	5	3	3	3	3	3	3	7	1	5	1	1	1	2	3
I 190	9	1	1	5	1	1	1	7	1	1	3	1	1	5	5	3	3	3	5	1	7	1	4	1	1	3	4	3
EL 1	1	1	1	5	1	1	1	7	9	5	5	1	1	5	5	5	3	5	3	1	5	1	5	1	1	3	4	3
EL 2	9	9	9	5	1	1	1	7	1	1	3	9	1	7	5	5	3	5	3	3	5	1	5	1	1	1	4	5
I 200	1	9	1	5	1	1	1	3	1	1	5	1	1	7	5	7	3	5	3	1	7	1	5	1	1	3	2	3
I 202	1	1	1	5	1	1	1	7	1	1	3	1	1	5	3	5	3	3	5	1	7	1	6	1	1	2	2	5
I 205	9	9	1	5	1	1	1	7	1	1	5	1	1	5	5	5	3	5	3	2	7	1	5	1	1	1	4	3

Genotype	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
I 206	9	9	1	5	1	1	1	7	1	1	3	1	1	3	5	3	3	3	3	1	7	1	5	1	1	3	2	3
I 169	9	9	9	5	1	9	9	3	9	5	3	9	1	7	5	7	5	5	3	3	5	1	5	1	1	1	2	5
I 188	9	9	1	5	1	1	1	7	1	1	5	1	1	5	5	5	3	5	3	3	5	1	5	1	1	1	4	3
CM 139	9	9	9	3	1	1	1	7	1	5	3	1	1	5	5	3	3	5	3	1	5	1	5	1	1	3	4	5
SE 533A	9	9	1	5	1	1	1	7	1	5	3	1	1	5	7	3	3	5	3	1	5	1	5	1	1	1	2	3
CML 32	1	9	9	5	9	1	1	7	1	1	3	9	1	5	3	5	3	3	3	1	5	1	5	1	1	3	2	3
SE 544	1	9	1	5	1	9	1	7	1	1	5	9	1	5	7	3	3	5	3	1	7	1	5	1	1	3	2	3
SE 547	9	9	9	3	1	1	1	7	1	1	3	1	1	7	5	3	3	5	3	3	5	1	5	1	1	1	2	3
SE 549	9	9	1	5	1	1	1	7	9	5	5	1	1	7	5	3	3	3	3	3	5	1	5	1	1	3	2	3
SE 557	1	9	9	5	1	1	1	7	1	1	5	1	1	5	3	5	3	3	3	3	5	1	5	1	1	3	2	3
LM 22	1	1	9	5	9	9	1	3	1	1	5	1	1	7	3	5	3	5	3	3	5	1	4	1	1	3	4	3
LM 23	9	1	1	5	1	9	1	7	1	1	5	1	1	7	5	5	3	5	3	1	5	1	4	1	1	1	4	3
SE 563	1	9	9	7	1	9	1	3	9	5	7	9	1	5	7	3	5	5	3	3	5	1	5	1	1	1	2	3
SE 567	9	9	1	5	1	1	1	7	1	9	5	1	1	7	3	5	3	5	3	3	5	1	5	1	1	1	2	3
SE 571	9	9	9	5	1	1	1	7	9	5	5	9	1	7	5	3	5	5	3	3	5	1	5	1	1	1	2	3
EML 57	9	1	1	5	9	9	1	3	1	1	5	1	1	3	3	3	3	5	3	1	7	1	5	1	1	3	4	3
SE 601	1	1	1	5	1	9	9	7	1	5	5	9	1	7	5	5	5	5	5	1	7	2	4	1	1	3	1	3
SE 603	9	9	1	5	1	9	1	7	1	5	5	1	1	7	5	5	3	5	3	3	5	1	5	1	1	1	2	3
SE 604	1	1	9	5	1	9	1	7	1	5	3	1	1	7	5	5	3	5	3	3	5	1	5	1	1	3	2	5
SE 607	9	1	9	5	9	9	9	7	1	1	3	1	9	3	5	3	7	5	3	1	5	1	5	2	1	3	2	3
SE 610	9	1	9	7	1	1	1	7	9	5	5	9	1	5	5	7	7	5	5	1	7	1	5	1	1	1	2	5
SE 613	1	1	9	5	9	9	9	7	1	5	3	1	9	3	5	5	7	5	3	3	5	1	7	1	1	1	2	3

Genotype	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
SE 616	9	1	9	5	1	9	1	7	1	1	5	9	1	7	3	7	5	5	3	1	5	1	5	1	1	1	4	5
I 183	9	1	9	5	1	1	1	3	1	1	5	1	1	5	3	3	3	3	3	3	7	1	5	1	1	3	2	5
SE 503	9	9	1	7	1	9	1	7	1	5	5	1	1	7	5	5	3	5	3	3	7	1	5	1	1	3	4	3
EML 282	9	9	9	5	9	1	1	7	1	1	5	1	1	5	3	5	3	5	3	1	7	1	4	1	1	3	4	5
EML 132	1	1	9	5	9	9	1	7	9	1	5	9	1	7	5	5	3	5	3	3	5	1	4	1	1	1	4	5
LM16 Zp-1	1	1	9	5	9	1	1	7	1	1	3	1	1	7	5	7	5	5	3	1	5	1	4	1	1	1	4	5
SE 616	9	9	9	3	1	9	9	7	9	5	3	9	9	7	5	7	7	5	3	1	7	1	4	1	1	1	4	5
EML 122	1	1	1	3	1	1	1	7	1	1	3	1	1	5	5	5	5	5	3	3	5	1	4	1	1	1	4	5
EML 240	1	1	9	5	1	9	1	7	9	5	5	1	1	7	3	3	3	3	3	1	7	1	4	1	1	3	4	5
EML 126	1	1	1	5	1	9	9	7	1	1	5	9	1	5	5	3	3	5	3	3	5	2	4	1	1	1	4	5
EML 285	9	1	9	7	9	9	1	3	1	5	5	9	9	7	3	5	3	5	3	3	5	2	4	1	1	1	4	5
EML 272	1	9	1	5	1	1	1	7	1	1	5	1	1	5	5	3	3	5	3	3	5	1	4	1	1	3	4	3
EML 283	9	9	9	5	1	1	1	7	1	1	5	1	1	5	5	3	3	5	3	1	7	1	4	1	1	3	4	5
LM 16	1	1	9	5	9	1	9	7	1	1	3	1	1	5	5	5	3	5	3	3	5	1	5	1	1	3	2	3
LM 20	9	9	1	5	1	9	9	7	9	9	5	1	1	7	5	3	7	5	3	3	5	1	5	1	1	1	4	5

(A: Angle between the leaf blade and stem, B: Altitude of leaf blade, C: Anthocyanin col of brace roots, D: Time of anthesis (50%), E: Anthocyanin at the base of the glume, F: Anthocyanin colouration at glumes except base, G: Anthocyanin colouration of anthers, H: Density of spikelet, I: Angle between main axis and lateral tassel, J: Tassel: Altitude of lateral branch, K: Time of silk emergence (50%), L: Anthocyanin colouration of silk, M: Anthocyanin colouartion of leaf sheath, N: Tassel : Length of main axis, O: Plant : Ear Placement, P: Plant Height : Inbreds, Q: Width of leaf blade, R: Ear length without husk, S: Ear diameter without husk, T: Ear shape, U: Number of rows of grains, V: Type of grain (flint/dent/semi dent), W: Colour of top of grain, X: Anthocyanin colouration of glumes, Y: Type of grain, Z: Row arrangement, AA: Kernel shape, AB: Kernel size (1000 kernel weight)

4.9.2 Analysis of Variance (ANOVA) for measurable DUS characteristics

The lines subjected to DUS characterization were sown in three replications in Randomized Block Design (RBD). All the measurable characteristics were recorded from six plants for each line and from each replication. An average reading was calculated and subjected to One factor analysis. Significant differences were observed among the inbred lines for all the measurable characters. Significant difference for replication was observed for cob length, plant height and kernel weight. ANOVA for eight measurable DUS characters have been presented in Table 4.28.

4.9.3 Descriptive Statistics for measurable DUS characters

Based on the mean data calculated for the measurable traits, maximum, minimum, critical difference, standard error and coefficient of variation were calculated and summarised in Table 4.29. Among all inbred lines, I172 recorded maximum cob length of 18.4cm while minimum was recorded for I 206 with cob length of 6.74 cm. PML 333 and SE 603 with 4.12 cm and 2.11 cm respectively recorded maximum and minimum value for cob diameter. With a tassel length of 38 cm, SE 547 recorded maximum and with 16.33 cm, I 108 recorded minimum value for tassel length. The plant height was recorded highest for PML 387 with 172.67 cm and lowest for I 231 with 93.33 cm. Earliest Silking was observed for I 108 (50 days) while late for PML 41 (62 days). With 10.57 cm, SE 610 showed maximum width of leaf blade and I 182 with 5.67 cm, showed minimum leaf width. The anthesis period was shortest for I 108 with 48 days and longest for PML 41 with 60 days of anthesis period. Maximum grain weight was recorded for PML 1230 with 340.62g/1000 grain weight, while minimum was for SE 601 with 117.01g/1000 grain weight. The data has been presented in the box plot in the Fig. 4.15 below.

Table 4.28: Analysis of Variance for measurable DUS characters

Source of Variation	d.f.	Mean Squares							
		Cob length	Cob Diameter	Tassel length	Plant height	Silking Days	Leaf blade width	Anthesis days	Kernel 1000grains
Replication	2	5.0645*	0.255 NS	0.4875 NS	75.8165*	2.0375 NS	0.0765 NS	2.2625 NS	16.5595*
Treatment	79	11.952*	0.457*	106.932*	1285.535*	48.881*	4.201*	47.615*	8491.393*
Error	239	1.177	0.072	0.905	17.677	2.379	0.026	2.895	112.560

* = significant at 5% probability level

Table 4.29: Descriptive statistics of lines for measurable DUS characters

	Cob length (cm)	Cob diameter (cm)	Tassel length (cm)	Plant height (cm)	Silking days (days)	leaf blade width (cm)	Anthesis days (days)	Kernel 1000grain (grams)
max	18.4 (I 172)	4.12 (PML 333)	38 (SE 547)	172.67 (PML 387)	62 (PML 41)	10.57 (SE 610)	60 (PML 41)	340.62 (PML 1230)
min	6.74 (I 206)	2.11 (SE 603)	16.33 (I 108)	93.33 (I 231)	50 (I 108)	5.67 (I 182)	48 (I 108)	117.01 (SE 601)
C.D.	1.752	0.434	1.536	6.787	2.490	0.260	2.747	17.126
SE(m)	0.626	0.155	0.549	2.427	0.891	0.093	0.982	6.125
SE(d)	0.886	0.219	0.777	3.433	1.259	0.132	1.389	8.663
C.V.	9.396	7.862	3.439	3.363	2.759	2.227	3.298	5.213

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)

(j)

(k)

(l)

(m)

(n)

(o)

(p)

(q)

(r)

(s)

(t)

(u)

(v)

(w)

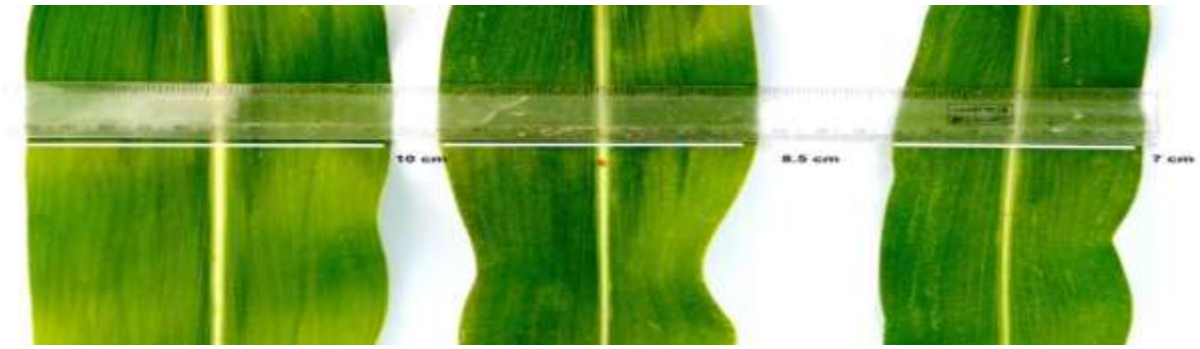
(x)

(y)

(z)

(aa)

(ab)



(ac)

Plate 4.7: Different visually assessed DUS characters observed at field condition.

Angle between stem and leaf blade a: acute, b: wide; Attitude of leaf blade c: droopy, d: straight; anthocyanin pigment e: present, f: absent; anthocyanin at base of glume g: present, h: absent; anthocyanin colouration of glumes i: present, j: absent; anthocyanin colouration of anthers k: present, l: absent; spikelet density m: dense, n: sparse; tassel angle o: normal, p: wide; anthesis of tassel q: straight, r: curved; anthocyanin colouration of silk s: present, t: absent; ear placement u: low and high, v: medium, tassel length w: short, x: medium, y: long; plant height z: short, aa: medium, ab: long; ac: different leaf width.

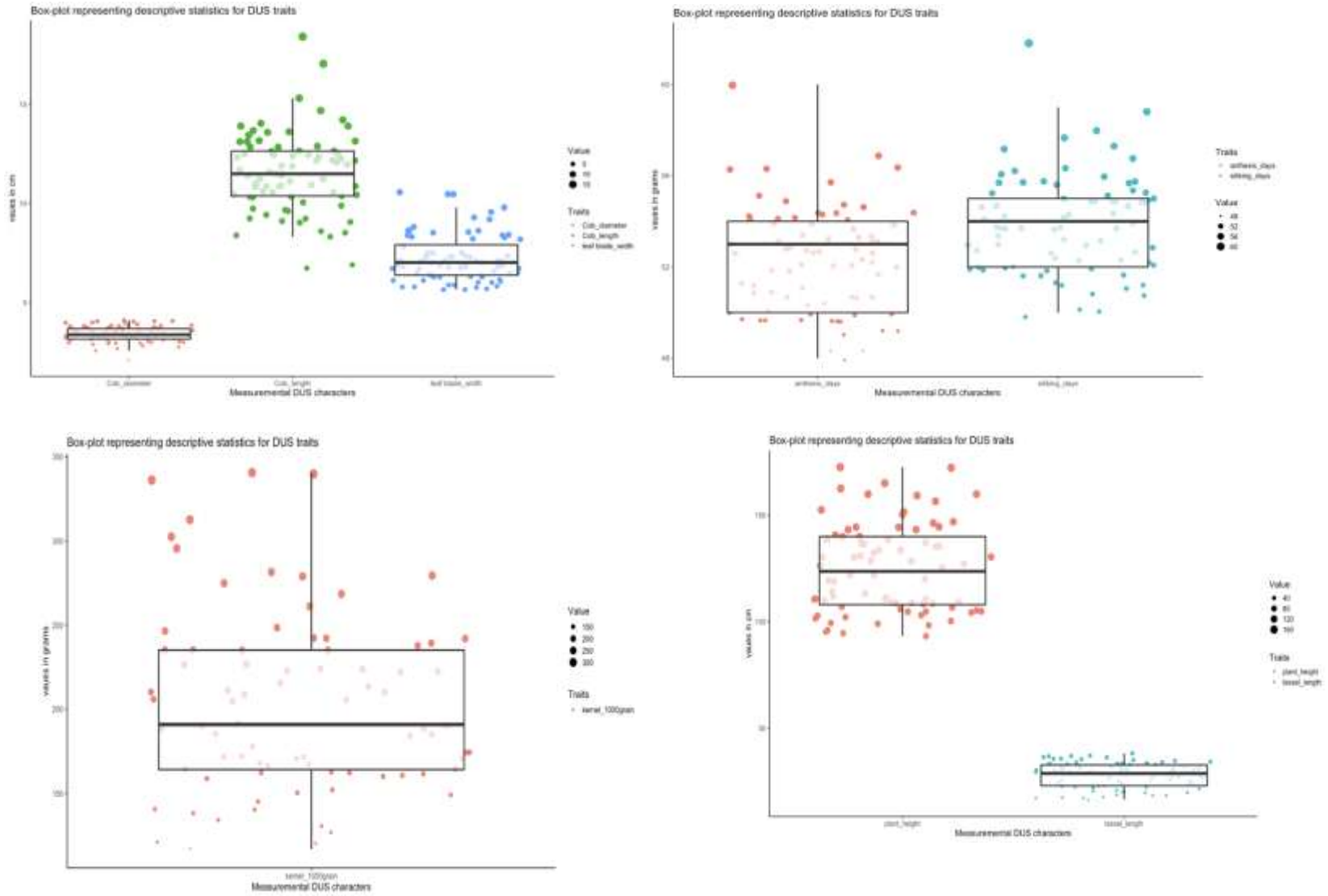


Fig. 4.15: Box plots showing distribution of genotypes based on 8 measurable DUS characters

4.9.4 Clustering and Grouping of the maize inbred lines

4.9.4.1 Clustering based on visual assessed characters:

Twenty different visually assessed DUS descriptors were employed to generate a Euclidian dissimilarity coefficient matrix followed by clustering using ward.D2 algorithm with the aid of RStudio software package. The dendrogram thus obtained from the analysis is presented in the Fig. 4.16. The dendrogram thus obtained revealed two major clusters. Both Cluster I and Cluster II were further divided into two sub-clusters. Cluster I included majority of genotypes consisiting of 54 lines while Cluster II included 26 genotypes. Each sub-cluster of Cluster I further divided into two major groups, while first sub cluster of Cluster II consisted of LM 21 and SE 607, and second sub cluster was bifurcated into two major groups.

4.9.4.2 Clustering based on measurable DUS characters

Eight different measurable DUS descriptors were used to generate a Euclidian dissimilarity coefficient matrix followed by clustering using ward.D2 algorithm with the aid of Rstudio. The circular dendrogram thus obtained from the analysis is presented in the Fig. 4.16. The cluster analysis clustered the 80 genotypes into two major clusters. Both clusters were further divided into two subclusters. Cluster I consisted of 23 genotypes while 57 genotypes were included in Cluster II. Each sub cluster further bifurcated into two major groups.

4.9.5 Correlation between several measurable DUS descriptors

Eight measurable DUS descriptors were subjected to correlation study based on mean values and thus obtained correlation coefficient between the traits were plotted using RStudio and thus obtained diagram is presented in Fig. 4.17. Positive and significant correlation was observed for silking and anthesis days, cob length and plant height and plant height and leaf blade width. Highest negative correlation was recorded for cob length and cob diameter. No significance was recorded for cob length with anthesis and silking days, tassel length with anthesis days, cob diameter and leaf blade width. Similarly anthesis days and leaf blade width, silking days and kernel weight, and plant height and silking days recorded no any significant correlation. Correlation study helps in predicating how one yield contributing factor is associated to another, and the study on mere fewer characters help in judging the performamce of other associated charcaters. Similar correlation between the measured traits has been recorded by the earlier findings (Aman *et al* 2020, Abadassi 2016, Khan *et al* 2018, Silva *et al* 2016).

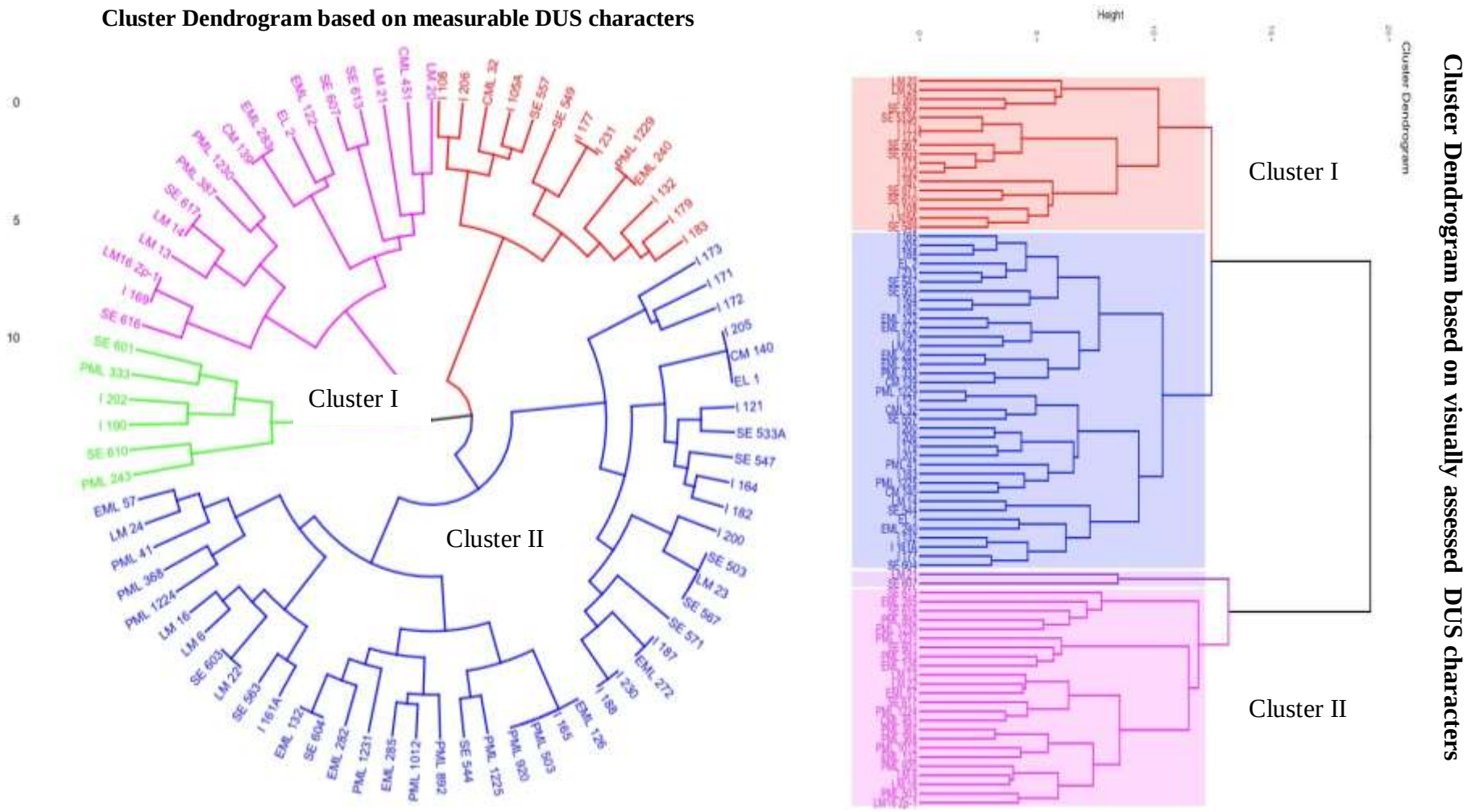


Fig. 4.16: Clustering of genotypes based on visually assessed and measurable DUS characters

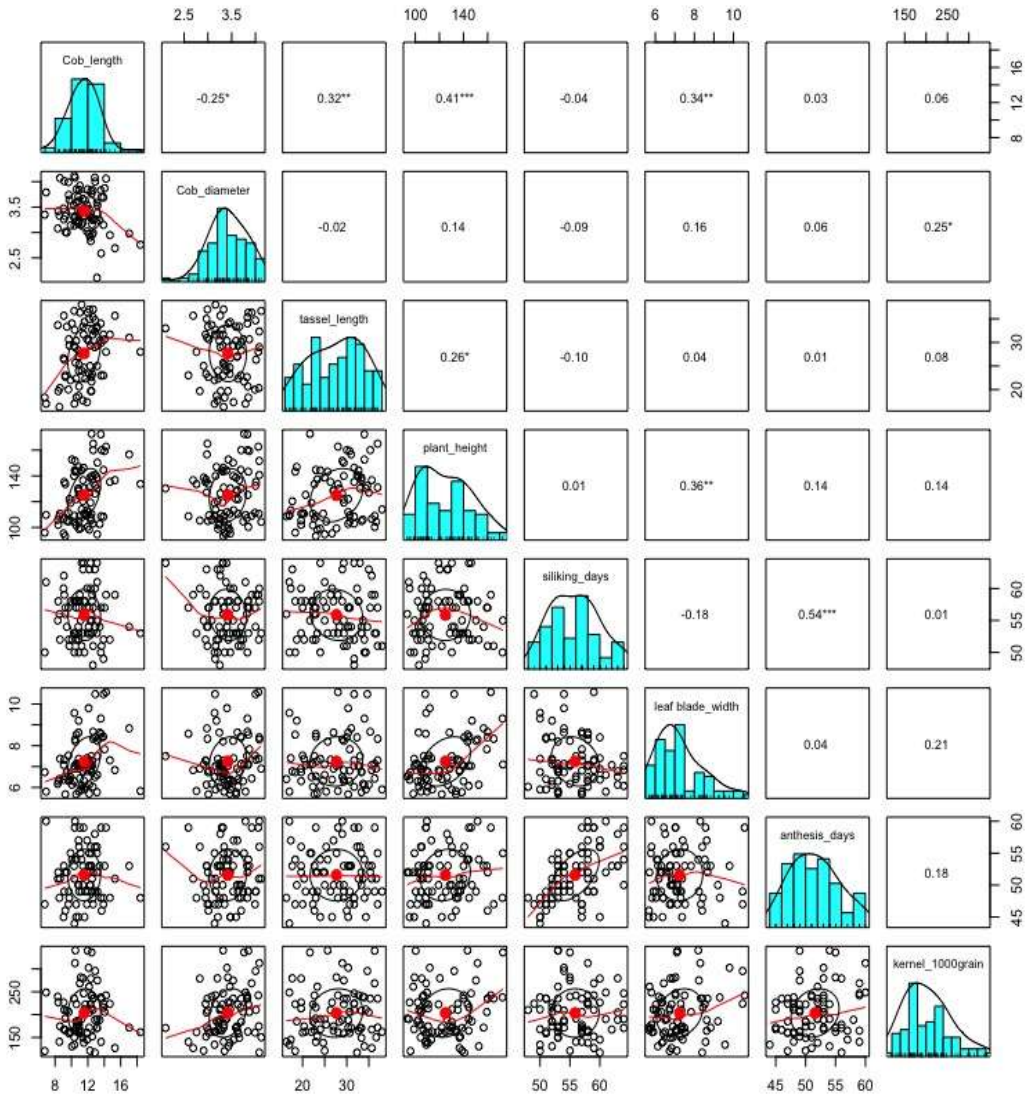
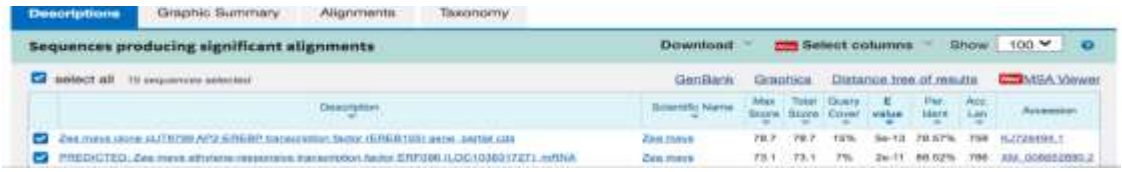


Fig. 4.17: Correlation between different DUS measurable characters.

4.10 Identification of orthologs for *Sub1A* and *SNORKEL* gene in maize

The sequence for *SNORKEL1* and *SNORKEL2* genes was retrieved from the NCBI database using the accession number AB510478 and AB510479 respectively (Hattori *et al* 2009). A complete CDS (1487 bp) of *Snorkel 1* gene from C9285 cultivar and complete CDS (1179 bp) of *Snorkel 2* gene from same C9285 cultivar of Indica group was retrieved from the database. Each gene has single CDS starting from 505 to 1275 bp for *Snorkel 1* and 126 to 902 bp for *Snorkel 2*. Both of the CDS produce ethylene response factor as product. These obtained CDS were BLAST run against B73 RefGen_V3 (taxid: 4577) reference genome. *Snorkel 1* showed no significant similarity against maize, while *Snorkel 2* showed some similarity against maize (15%), but since the similarity was non-significant results were discarded (Fig. 4.18).

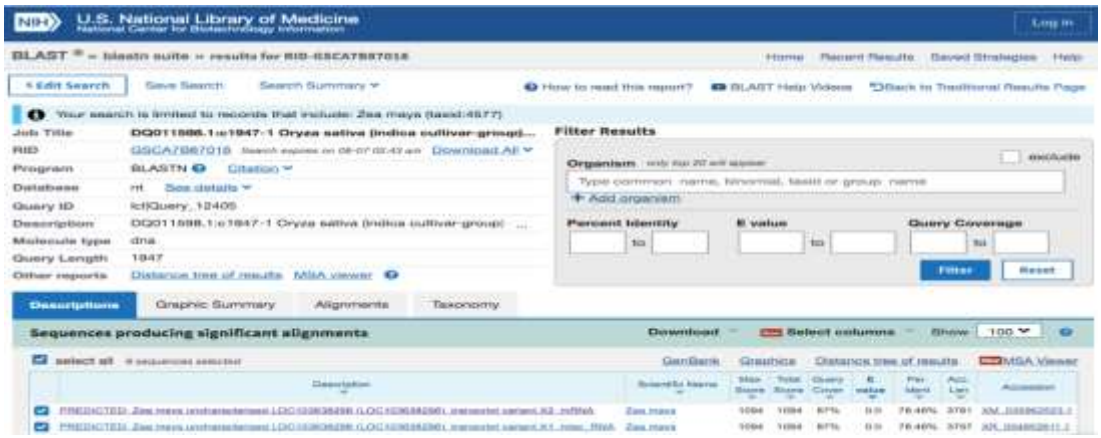
(A)



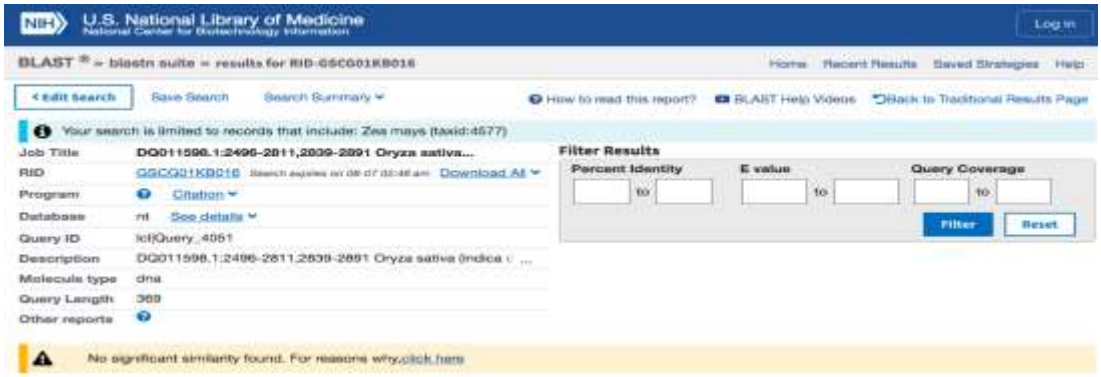
(B)

Fig. 4.18: A- *Snorkel 1* with no significant similarity when BLAST run against B73 RefGen_V3,B- *Snorkel 2* with 15% query cover when BLAST run against B73 RefGen_V3

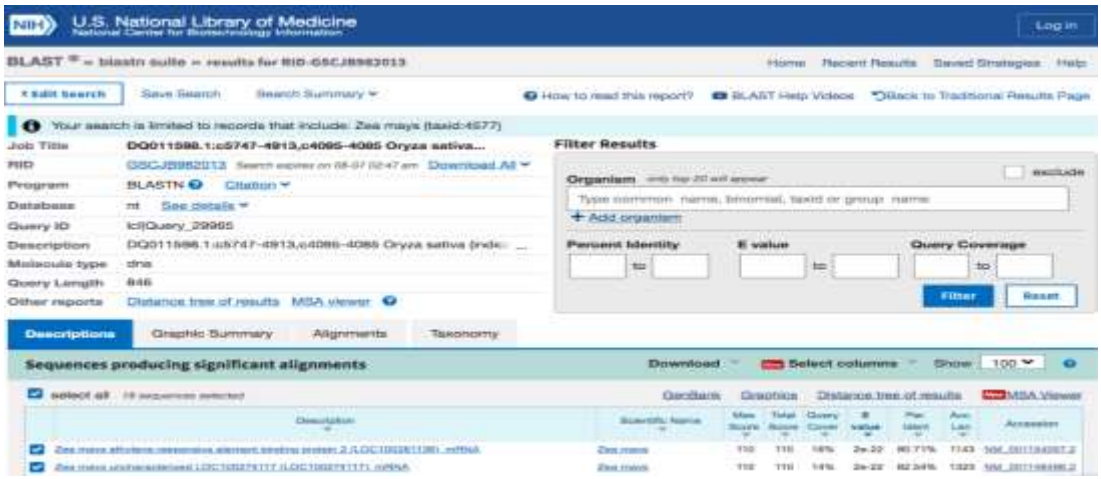
The sequence for *Sub1A* gene was retrieved from the NCBI database using the accession number DQ011598.1 (Singh *et al* 2020). Three CDS regions were obtained from the retrieved sequence, named as CDS1, CDS2 and CDS3. Among three CDS, CDS1 and CDS3 showed some level of similarity when these sequences were used as query against the maize database for ortholog sequence identification. Reference sequence of B73 RefGen_V3 (taxid: 4577) was used for orthologs identification using Nucleotide BLAST available in NCBI BLAST. A complete sequence (7355 bp) from sub clone 26D17-13 of cultivar IR40931-26 from indica cultivar group of rice was retrieved. CDS1 extends from 1 to 1947 bp, CDS2 from 2496 to 2811 and 2839 to 2891 with an intron in between and CDS3 extends from 4085 to 4095 and 4913 to 5747. CDS1 yields transposase protein, CDS2 produces hypothetical protein and CDS3 produces *sub1A-1* protein which is considered to be major ERF for conferring tolerance to submergence in rice. Individual CDS was BLAST run against maize reference genome keeping all the other parameters as default and result showed no similarity for CDS2, while CDS1 and CDS3 showed 87% and 16% query cover with percent identity of 78.46% and 80.71% respectively. The results are shown in the Fig. 4.19 below.



(A)



(B)



(C)

Fig. 4.19: A: *Sub1 CDS1* showing 87% query cover with 78.46% identity, B: *Sub1 CDS2* with no significant similarity, C: *Sub1 CDS3* showing 16% query cover with 80.71% identity

4.11 Primer designing

Two orthologs sequence for *Sub1A CDS1* and *Sub1A CDS3* were used to design the gene specific primers. The ortholog genes XM_035962523.1 for CDS1 and NM_001154057.2 for CDS3 found in maize showing sequence similarity were then subjected to Primer3 online primer designing application to design gene specific primers. A list of primers was obtained from Primer3, and the most appropriate primer was selected keeping in mind that both forward and reverse for each primer falls within approximate same melting temperature, chances of primer dimer formation were avoided, care taken to assure no hair-pin structure were formed. Finally, the G/C content of the primer was kept around 50% and primer synthesis was done by outsourcing. Primer designed from the orthologs are presented in Table 4.30.

Table 4.30: Gene specific primers designed for genotyping using maize orthologs

Primers		Sequence	Length	Tm	%GC	Product size
Sub1A CDS 1 Maize ortholog (XM_035962523.1)	Forward	GAAGGCATTGATGACGACCT	20 bp	52	50	152 bp
	Reverse	CCCTCAACCATGACTCCACT	20 bp	54	55	
Sub1A CDS 3 Maize ortholog (NM_001154057.2)	Forward	GACCTCGAGGAACAAGAGCA	20 bp	54	55	239 bp
	Reverse	AAGAGGAGCGAGTCGAACAG	20 bp	54	55	

4.11.1Detection of allelic variation

Ninety-five diverse maize lines including one accession of *Z. mays spp parviglumis* were used to detect the allelic variation, if any, for the candidate genomic region by designing the gene-based markers. Two gene specific markers designed earlier for CDS1 and CDS3 orthologs were employed for the same. Standard PCR protocols were followed and the PCR amplicons were run in Agarose gel to detect polymorphism amongst the experimental materials.

CDS1 showed the good amplification with an amplicon size of 152 bp, but no polymorphism was obtained suggesting no sequence variation or very minor undetectable sequence variation in the genotyped inbred lines (Plate 4.8).

CDS3 showed non-specific binding and clear observation was not detected (Plate 4.9).

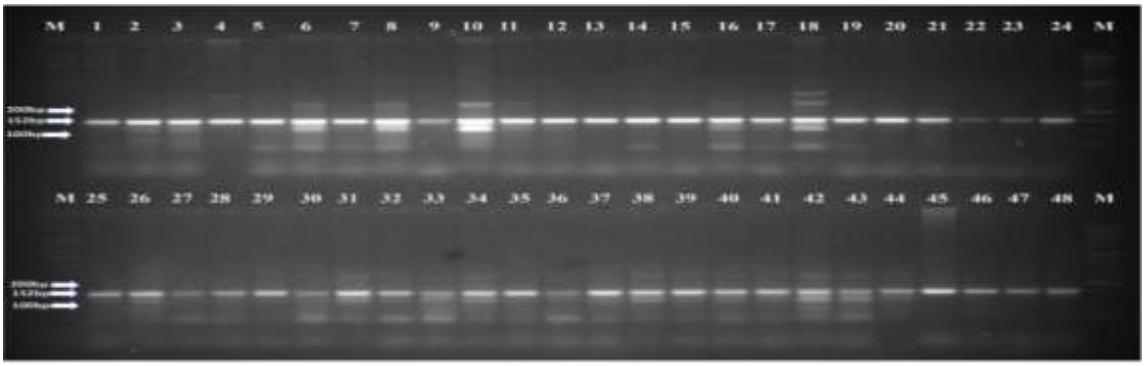
The gene specific primers used for the current study to detect the allelic variation for *Sub1A* ortholog in Maize did not give desired output. Occurance of monomorphic band size revealed absence of significant sequence variation. This study showed no sequence variation for this gene across the diverse genotypes of maize exhibiting varying degree of tolerance

against waterlogging stress. This primary study suggested that there was no involvement of these genes in conferring tolerance in maize. The obtained result might be due to presence of very minimal difference in their sequence or the region being conserved across the genotypes. To further elucidate the involvement of these orthologs, gene expression analysis was carried out. Genotypes showing extreme phenotypes were included in the study and RT-PCR (Real time Polymerase Chain Reaction) was carried out to study the same.

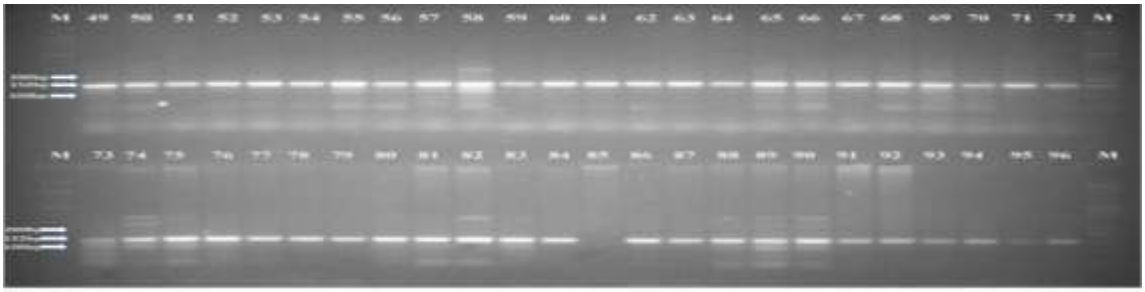
List of 96 inbred (including one *Z. mays spp parviglumis*) used in the experiment are enlisted in Table 4.31.

Table 4.31: List of 96 genotypes used for detection of allelic variation

1	I 164	**25**	I202	**49**	SE571	**73**	EML279
2	EML122	**26**	I205	**50**	SE601	**74**	EML124
3	EML285	**27**	PML1241	**51**	PML387	**75**	PML384
4	VL1010764	**28**	PML1225	**52**	SE616	**76**	LM13
5	SE 603	**29**	PML920	**53**	I165	**77**	AVL13119
6	CM140	**30**	LM19	**54**	EML52	**78**	LM26
7	CML32	**31**	PML898	**55**	SE604	**79**	CM139
8	CML451	**32**	NL219605	**56**	SE503	**80**	LM5
9	EML57	**33**	I200	**57**	EML53	**81**	PML333
10	PML1230	**34**	AVM11207	**58**	I189	**82**	LM16
11	I121	**35**	LM23	**59**	LM6	**83**	SE544
12	I132	**36**	PML169	**60**	I168	**84**	LM11
13	161A	**37**	PML224	**61**	SE607	**85**	LM17
14	I206	**38**	I162	**62**	I179	**86**	LM12
15	I169	**39**	PML1012	**63**	I192	**87**	CHT219301
16	I170	**40**	I177	**64**	I190	**88**	JCY20
17	I171	**41**	PML1229	**65**	EML125	**89**	PML368
18	I172	**42**	I105A	**66**	EML130	**90**	LM22
19	I173	**43**	PML243	**67**	EML240	**91**	PML373
20	PML1228	**44**	SE610	**68**	PML400	**92**	EML126
21	I185	**45**	PML503	**69**	I204	**93**	AVL13056
22	EML128	**46**	SE533A	**70**	EML283	**94**	PML28
23	EML282	**47**	SE563	**71**	EML132	**95**	PML224
24	LM20	**48**	SE567	**72**	EML272	**96**	*Z. mays spp parviglumis*

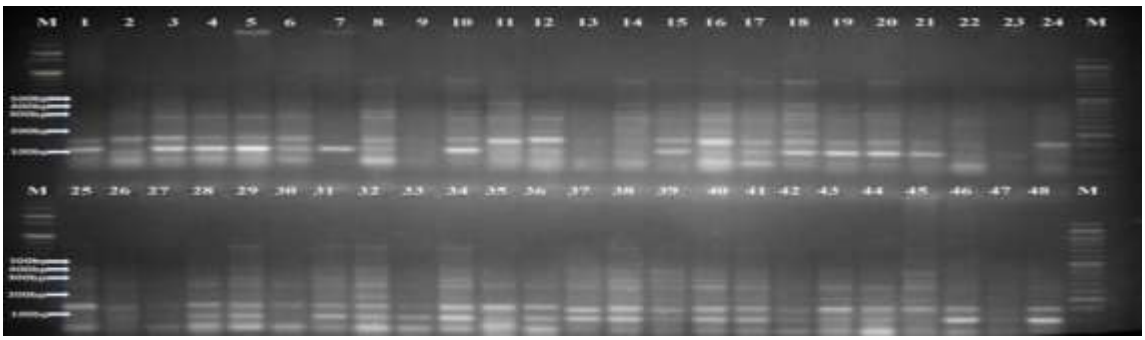


(A)

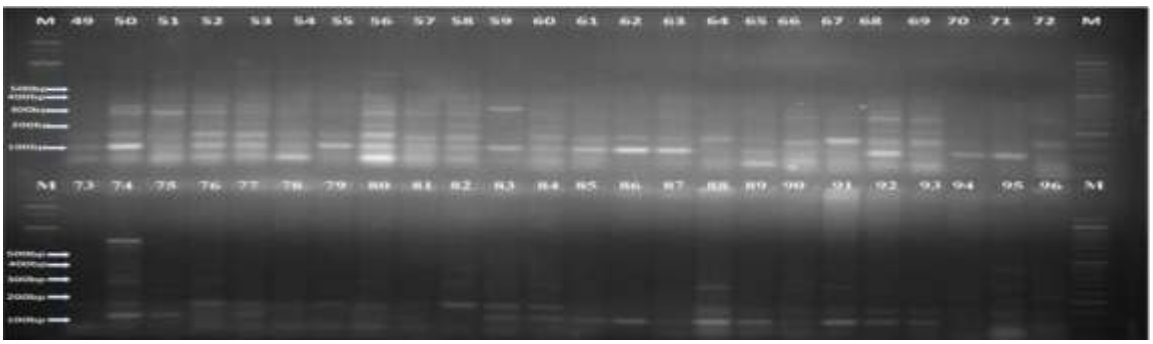


(B)

Plate 4.8: A- Gel image of *SUB1ACDS1* ortholog showing amplicon size of 152bp run on 1-48 inbred lines, B – Gel image of *SUB1ACDS1* ortholog showing amplicon size of 152bp run on 49-96 inbred lines. M- gene ladder of 100bp



(A)



(B)

Plate 4.9: A- Gel image of *SUB1A CDS3* ortholog showing non-specific amplicon run on 1-48 inbred lines, B – Gel image of *SUB1A CDS1* ortholog showing non-specific amplicon run on 49-96 inbred lines. M- gene ladder of 100bp

4.12 RNA extraction

4.12.1 Sample collection

Genotypes showing extreme response against waterlogging stress along with one accession of *Z. mays spp parviglumis* were selected based on the phenotypic observation and these lines were used for the RNA extraction and further gene expression analysis. Root samples from each of the five lines were collected at three different duration of waterlogging, *viz*. 3 days of continuous waterlogging, 6 days of continuous waterlogging and 9 days of continuous waterlogging. Samples from the control set was also harvested at the corresponding duration. Standard protocol of RNA extraction was followed and quality checked at both Nanodrop spectrophotometer and Agarose gel in MOPS buffer. Tolerant lines (I 168 and I 172), two susceptible lines (I 200 and PML 898) and *Z. mays spp parviglumis* were selected and RNA extraction was carried out in 3 different duration of waterlogging.

RNA extraction for 3 days of control and 3 days of waterlogging were extracted and checked over agarose gel and picture captured using gel documentation system (Plate 4.10). Total 30 samples were collected from 6 different treatments that is 3 control and 3 waterlogging and thus collected RNA were used for further analysis.

4.12.2 Gene expression study

RNA extraction was done from the root samples collected from the lines subjected to waterlogging for 3 days, 6 days and 9 days, and corresponding samples from the control plot. Tolerant and susceptible lines were selected based on the survival data, and other phenotypic observations. Quality and quantity of the RNA was checked using nanodrop spectrophotometer and denaturing agarose gel images and thus obtained pure high-quality RNA was used to make cDNA following the manufacturers' protocols.

Two gene specific primers were designed from the orthologous gene in maize obtained from the BLASTn result (Table 4.32) when the Submergence 1A (*Sub1A*) was run as query sequence against maize reference genome. Among three coding sequence (CDS) of *Sub1A*, two CDS showed some level of similarity and these regions were used to develop gene specific primers. *Sub1A* CDS1 from *O. sativa* showed significant similarity with *Predicted: Zea mays uncharacterized LOC103938296.transcript variant X2.mRNA* from maize and *Sub1A* CDS3 showed similarity with *Zea mays ethylene-responsive element binding protein 2 (LCO100281138).mRNA*. These genes were used to generate gene specific primers and expression analysis was carried out using RT-PCR (Real Time Polymerase Chain Reaction) in StepOnePLus^{TM} Real-Time PCR system. Takara TB Green Premix Ex Taq dye was used to measure the relative expression level of the gene. As an internal control 18s gene was used and normalization was carried out.

Table 4.32: List of primers along with 18s as internal control used for gene expression

Primers		Sequence
Sub1A CDS 1 Maize ortholog (XM_035962523.1)	Forward	GAAGGCATTGATGACGACCT
	Reverse	CCCTCAACCATGACTCCACT
Sub1A CDS 3 Maize ortholog (NM_001154057.2)	Forward	GACCTCGAGGAACAAGAGCA
	Reverse	AAGAGGAGCGAGTCGAACAG
18s	Forward	AGTTTGAGGCAATAACAGGTCT
	Reverse	GATGAAATTTCCCAAGATTACC

Three technical replicates were used for the detection of the RT value and an average value was calculated for the analysis. A melt curve obtained from the analysis is shown in Fig. 4.20.

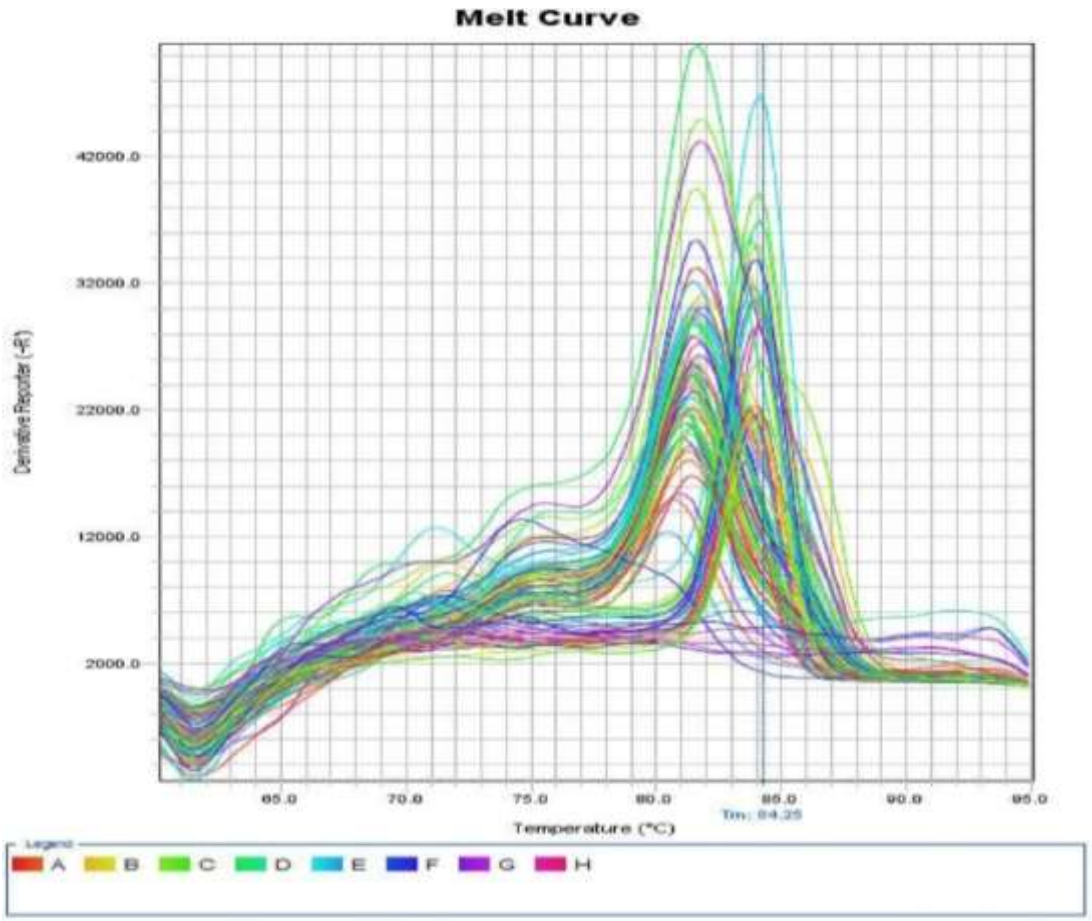


Fig. 4.20: Melt curve showing the melting temperature for the primer under investigation (*Sub1A CDS1* ortholog) and control primer

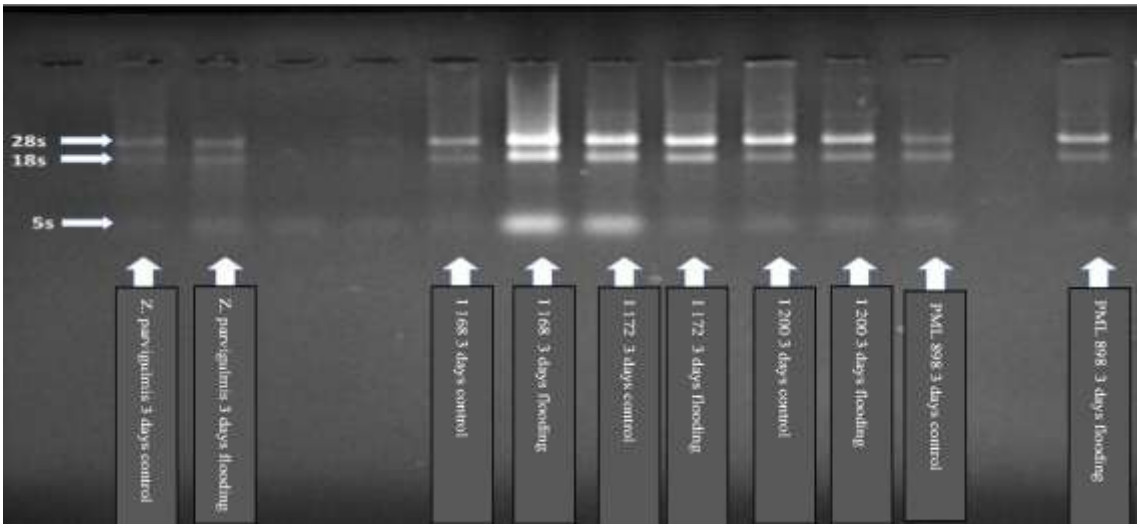


Plate 4.10: RNA quantification over agarose gel for samples collected at 3 days control and 3 days of waterlogging

4.12.3 Expression changes in different lines

4.12.3.1 *Sub1 CDS1* ortholog gene

Two tolerant lines (I 168 and I 172) and two susceptible lines (I 200 and PML 898) along with one accession of *Z. mays spp parviglumis* were used for the expression analysis of two primers. Results from the study showed that almost all the lines taken in the study has increased level of expression in early waterlogged condition, when the water stagnation was for 3 days, as compared to control condition. Though the expression increased in all the lines, genes under study were highly expressed in the tolerant lines in comparison to both the susceptible lines and *Z. mays spp parviglumis*. The fold change increase in expression level was 12.53 times and 7.38 time in tolerant lines while it was 2.85 and 0.81 in susceptible lines and 1.93 in *Z. mays spp parviglumis* (Table 4.33 and Fig. 4.21). But as the duration of waterlogging stress lengthen, the respective fold change value drops drastically. Almost all the lines showed decrease in fold change value in both, tolerant and susceptible lines and in *Z. mays spp parviglumis*. But as the waterlogging stress duration increased from 6 to 9 days, slight increase in the expression level was seen but the *Z. mays spp parviglumis* showed drastic enhancement in the expression and the fold change was reported to be 5.19. The result depicted that the high expression of *Sub1 CDS1* ortholog during early stage in tolerant maize line may help plants to adjust themselves to the stress condition and thus providing them with much tolerance as compared to susceptible lines. Over expression of gene in *Z. mays spp parviglumis* in later stage of waterlogging may be the reason for *Z. mays spp parviglumis* to be highly tolerant against waterlogging for a prolonged duration.

4.12.3.2 *Sub1 CDS3* ortholog gene

Sub1 CDS3 ortholog also showed a similar trend in the gene expression as *Sub1 CDS1* gene ortholog. This gene was highly upregulated in tolerant lines with the fold change of 15.85 in I 172 and 2.23 in I 168 during early waterlogging stress, while this gene was downregulated in susceptible lines with a fold change of 0.64 and 0.72. *Z. mays spp parviglumis* showed slight upregulation with a fold change of 1.72. While during 6 days after waterlogging no significant gene expression was found in stressed condition as compared to control except for susceptible line PML 898, where the gene expression increased significantly and fold change was 6.26. On the 9 days after waterlogging the gene expression again showed increasing trend (Table 4.34 and Fig. 4.22). These results also showed that over expression of this gene at early stage of waterlogging is responsible for determining the tolerance in plants. Though the expression of this gene has significantly improved at 6 days of waterlogging condition in susceptible line but still the plant exhibited the susceptible reaction proving early expression of this gene is necessary for incurring tolerance in the plants.

Table 4.33: CT values and fold changes of *Sub1 CDS1* ortholog in maize lines (I 172, I 168, I 200, PML 898) at different duration of waterlogging

Lines	3 days of waterlogging		6 days of waterlogging		9 days of waterlogging		Fold change		
	control	treatment	control	treatment	control	treatment	3 days	6 days	9 days
I 172	21.58±0.69	21.73±0.31	23.68±022	22.32±0.23	22.02±1.71	22.30±0.54	12.53	0.34	0.5
I 168	21.41±0.75	22.49±0.40	20.22±0.29	21.4±0.40	20.96±0.09	21.51±0.73	7.38	1.17	1.04
I 200	22.85±0.07	23.73±0.98	22.50±0.38	21.73±0.23	22.21±0.39	22.49±0.23	2.85	0.014	0.38
PML 898	23.06±0.60	23.73±0.36	24.63±0.97	24.75±0.19	23.03±0.78	21.98±0.13	0.81	2.02	1.1
Z. mays spp. parviglumis	21.35±0.44	22.48±0.24	22.29±0.69	21.83±0.59	22.27±0.71	20.89±0.14	1.93	0.45	5.19

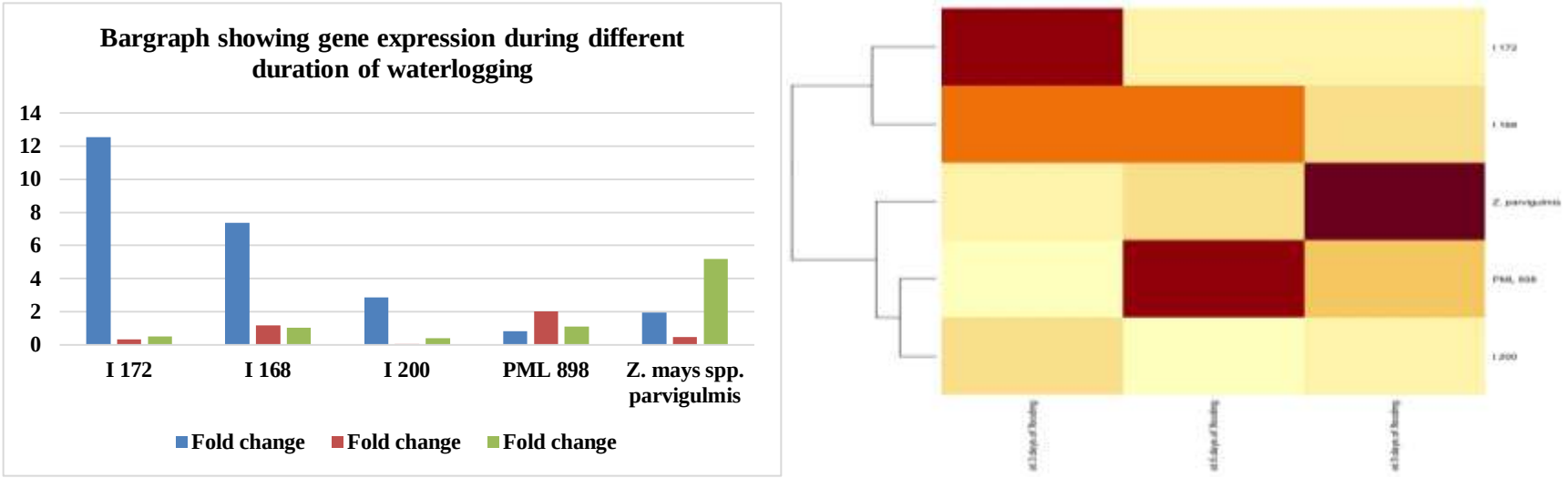


Fig. 4.21: Bar graph and heat map showing expression of *Sub1 CDS1* ortholog in maize lines (I 172, I 168, I 200, PML 898) along with *Z.mays spp parviglumis* at different duration of waterlogging

Table 4.34: CT values and fold changes of *Sub1 CDS3* ortholog in maize lines (I 172, I 168, I 200, PML 898) at different duration of waterlogging

Lines	3 days of waterlogging		6 days of waterlogging		9 days of waterlogging		Fold change		
	control	treatment	control	treatment	control	treatment	3 days	6 days	9 days
I 172	25.53±0.36	26.01±0.15	25.92±0.01	23.68±1.06	25.77±0.32	25.67±0.20	15.85	0.18	0.39
I 168	26.82±0.15	26.17±0.06	25.83±0.96	23.20±0.62	25.13±0.69	26.83±0.20	2.23	0.017	2.32
I 200	26.14±0.35	24.88±0.12	24.89±0.03	25.69±0.43	25.77±0.24	25.44±0.40	0.64	0.044	0.25
PML 898	25.74±0.02	26.25±0.54	25.08±0.98	26.83±0.52	26.23±0.26	26.63±0.23	0.72	6.26	3.02
Z. mays spp. parviglumis	24.79±0.15	25.76±0.31	26.47±0.45	26.31±0.33	26.23±1.21	23.33±0.28	1.72	0.56	1.71

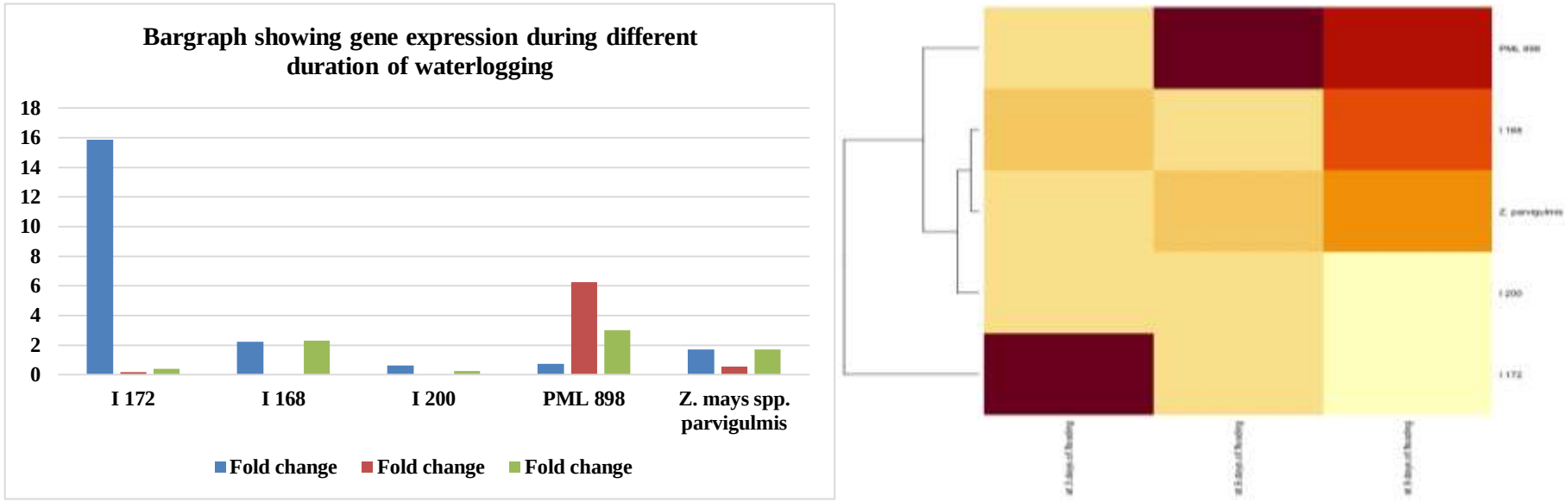


Fig. 4.22: Bar graph and heat map showing expression of *Sub1 CDS3* ortholog in maize lines (I 172, I 168, I 200, PML 898) along with *Z. mays spp parviglumis* at different duration of waterlogging

4.13 Population structure analysis

Twenty polymorphic SSR markers obtained from the previous study were employed for the genotyping of 96 maize inbred lines and these 20 markers yielded 77 different polymorphic loci. The results were analyzed in Gel Documentation System and the scoring of the data was done in such a way that the presence of the band was scored as "1" and the absence as "0". Thus, obtained data were then subjected to population structure analysis using STRUCTURE 2.3.4 software package (Pritchard *et al* 2000) employing admixture model-based clustering method. The parameter for running length was set as length of burn-in period as 10,000 and number of Markov Chain Monte Carlo (MCMC) reps after burn-in as 10,000. Different combinations of k value (number of clusters) with different alterations were checked and a final model was created by setting number of clusters (k = 10) with 10 different alterations for each K. To find the best K among all the obtained values, the whole data was compressed in a ".zip" file and was uploaded into the STRUCTURE HARVESTER database utilizing the Evanno method (Evanno *et al* 2005). The results obtained are summarized in the Table 4.35 below. Maximum DeltaK value was obtained for k=3, was the best K for our analysis. The Evanno table output and the bar plot for the population structure at k = 3 is presented in Fig. 4.23.

Table 4.35: List of DeltaK value generated from STRUCTURE HARVESTER database utilizing the Evanno method

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	DeltaK
1	10	-7091.210000	1.589864	—	—	—
2	10	-6466.380000	1.671859	624.830000	101.810000	60.896299
3	10	-5943.360000	2.666333	523.020000	183.850000	**68.952370**
4	10	-5604.190000	39.660236	339.170000	150.580000	3.796750
5	10	-5415.600000	16.770145	188.590000	28.900000	1.723300
6	10	-5255.910000	11.568679	159.690000	2.450000	0.211779
7	10	-5093.770000	13.898445	162.140000	0.550000	0.039573
8	10	-4931.080000	18.017818	162.690000	25.360000	1.407496
9	10	-4793.750000	14.394308	137.330000	20.630000	1.433205
10	10	-4677.050000	16.118261	116.700000	—	—

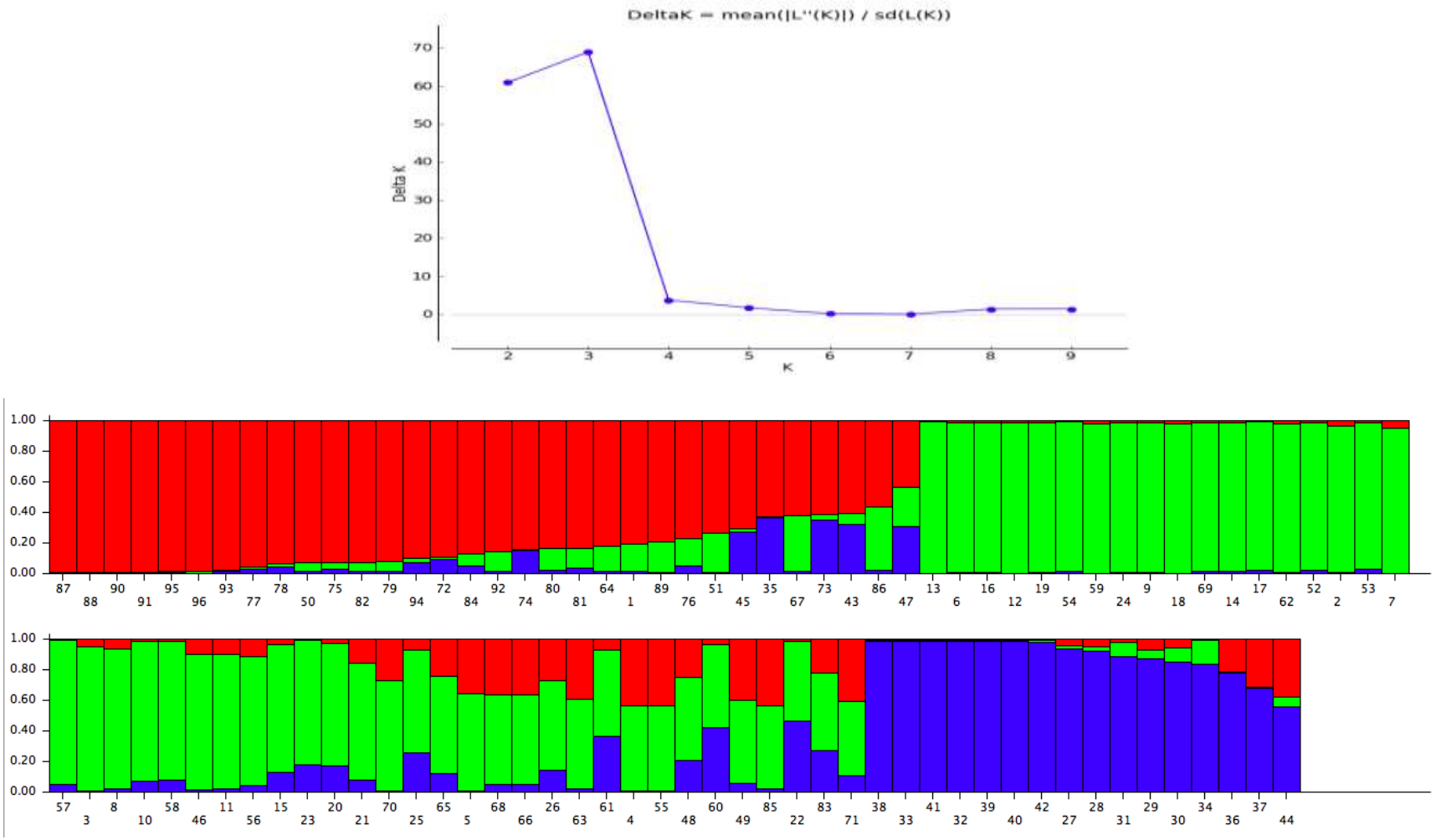


Fig. 4.23: Line graph showing highest DeltaK at k=3 and Bar graph showing distribution of different population

4.14 Genetic diversity and Polymorphism Information Content (PIC)

Twenty SSR markers were employed for the genotyping of 96 maize inbred lines, among which all the markers showed varying level of polymorphism. The polymorphic band obtained from the genotyping were scored and thus obtained result was analysed through Power Marker v 3.25 (Liu and Muse 2005). The heterozygosity varied from 0 (bnlg2199, umc1383, umc2210) to 0.9896 (umc1021) with an average of 0.3367. A total of 83 allelic loci were generated from 20 different markers with an average of 4.15 loci per marker. The PIC ranged from 0.2289 for umc2210 to 0.7530 for bnlg244 with an average of 0.503. Major allele frequency ranged from 0.8593 (bnlg1812) to 0.302 (bnlg2440) with an average value of 0.557. A descriptive summary from the analysis has been presented in Table 4.36.

4.15 Cluster analysis of genotypes based on genotyping data

The genetic relationship between the genotypes based on the genotypic data was analysed using Power marker v 3.25 where the genetic distance between the genotypes were analysed using Nei 1983 method. Thus obtained model was used for phylogeny study and dendrogram construction with Neighbour Joining (NJ) model. The tree was constructed with an aid of MEGA 7 software (Kumar *et al* 2016). 96 genotypes based on 20 SSR markers were clustered into two major clusters. Cluster I is subdivided into two sub clusters including 24 and 28 genotypes in each subclusters. Cluster II is again subdivided into two sub clusters. First subcluster of Cluster II included SE 610, SE 533A, SE 567 and I 179, while second sub cluster of Cluster II includes 40 genotypes. Since all the markers used in the current study were used selected on the basis of earlier study showing polymorphism for waterlogging associated traits. Genotypes are clustered accordingly.A dendrogram obtained from the study is shown in Fig. 4.24. Clustering of 96 genotypes based on 20 SSR markers assigned for waterlogging grouped them based on their tolerance against waterlogging. Most tolerant lines get clustered in one sub-cluster while the susceptible one gets into another.

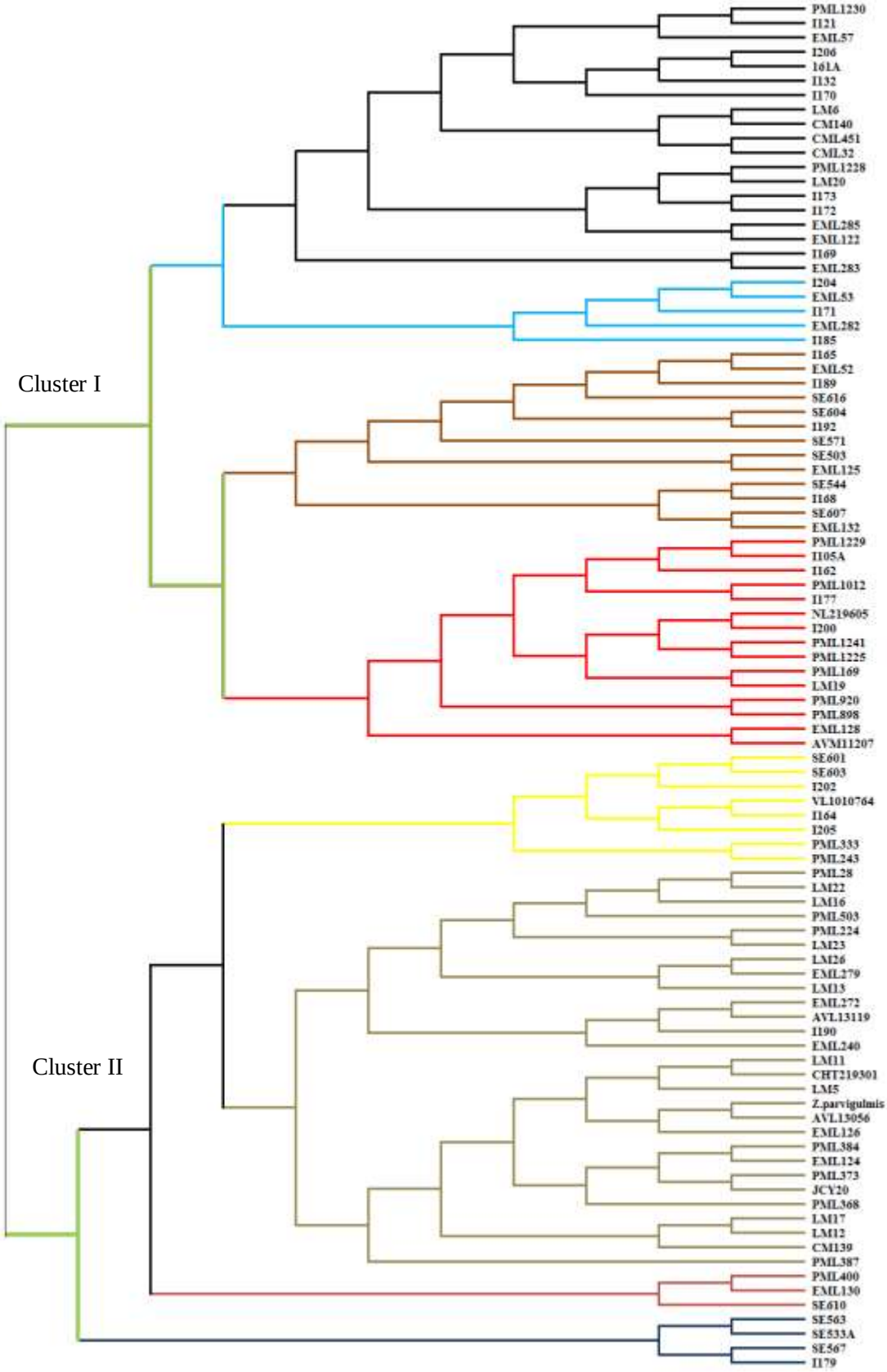


Fig. 4.24: Clustering of 96 genotypes based on genotyping following Neighbour Joining (NJ) method

Table 4.36: Gene diversity, Heterozygosity and Polymorphism Content (PIC) value for 20 SSR markers

Marker	Major Allele Frequency	Genotype No.	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Heterozygosity	PIC
bnlg244	0.302083333	18	96	96	8	1	0.783420139	0.21875	0.753094452
umc1236	0.583333333	6	96	96	4	1	0.575032552	0.239583333	0.513810356
umc1782	0.4375	5	96	96	3	1	0.6328125	0.885416667	0.555633545
umc1676	0.770833333	4	96	96	3	1	0.376898872	0.302083333	0.342256227
umc2340	0.491071429	12	96	56	8	0.583333333	0.668845663	0.375	0.621788765
umc2134	0.763157895	4	96	95	4	0.989583333	0.366315789	0.126315789	0.306584857
umc1033	0.41011236	15	96	89	6	0.927083333	0.742772377	0.393258427	0.707519857
umc2199	0.479166667	3	96	96	3	1	0.633897569	0	0.562134896
bnlg1812	0.859375	6	96	96	4	1	0.252278646	0.03125	0.238653209
umc1383	0.747368421	2	96	95	2	0.989583333	0.377617729	0	0.306320154
bnlg1007	0.515625	7	96	96	4	1	0.624674479	0.416666667	0.561906558
umc2204	0.5	6	96	95	4	0.989583333	0.623102493	0.378947368	0.552697126
bnlg182	0.5	7	96	94	4	0.979166667	0.591896786	0.287234043	0.509489529
umc2227	0.463157895	5	96	95	4	0.989583333	0.602271468	0.252631579	0.518717989
phi001	0.542105263	10	96	95	4	0.989583333	0.635069252	0.6	0.590055956
bnlg1016	0.531914894	4	96	94	3	0.979166667	0.566263015	0.180851064	0.478595833
umc1021	0.494791667	3	96	96	3	1	0.551269531	0.989583333	0.450109645
umc2210	0.84375	2	96	96	2	1	0.263671875	0	0.228910446
umc2048	0.478494624	11	96	93	6	0.96875	0.684472193	0.365591398	0.63996219
umc1052	0.377659574	5	96	94	4	0.979166667	0.686849253	0.691489362	0.625691698
Mean	0.554575034	6.75	96	92.95	4.15	0.968229167	0.561971609	0.336732618	0.503196664

4.16 Analysis of Molecular Variance (AMOVA)

The sub-population obtained from the STRUCTURE were further used to determine the genetic distance between these individuals using GenAlEx6.52b2 (Peakall and Smouse 2006). AMOVA was calculated to know the amount of variation present among population, among individuals and within individuals. Summery from the GenAlEx6.52b2 is presented in Table 4.37. F-statistics from the above analysis is summarized in the table below. Fst value of 0.087 revealed moderate genetic difference. The within-population inbreeding coefficient (F_{IS}) is 0.402 and inbreeding coefficient determined for the total population (F_{IT}) per locus is 0.454.

Table 4.37: Analysis of Molecular Variance between different sub-populations

Source	df	SS	MS	Est. Var.	%
Among Pops	2	76.109	38.055	0.519	9%
Among Individuals	93	709.073	7.624	2.187	37%
Within Individuals	96	312.000	3.250	3.250	55%
Total	191	1097.182		5.956	100%

F-Statistics	Value	P(rand >= data)
Fst	0.087	0.001
Fis	0.402	0.001
Fit	0.454	0.001

4.17 PCoA (Principal Coordinates Analysis)

To graphically visualize the resemblance between the individuals, PCoA was carried out in GenAlEx6.52.b2, an add-ons in excel, which clustered the genotype based on the similarity and dissimilarity. Individuals ordinated closure are more similar than those ordinated at further distance. The major variation were explained by first 3 axes with 11.70, 9.88 and 6.63 % variation with a cumulative of 28.22%. Results represented graphically in Fig. 4.25.

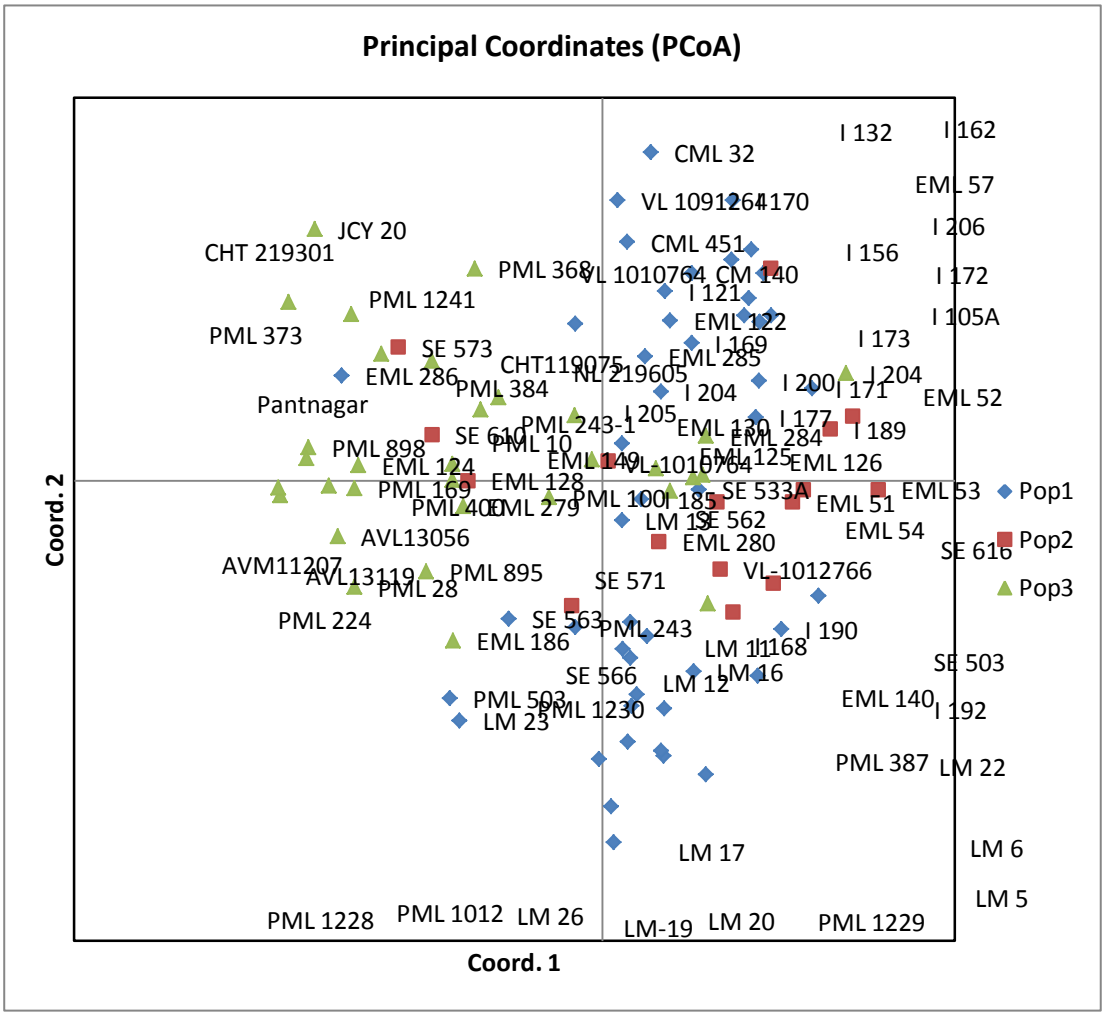


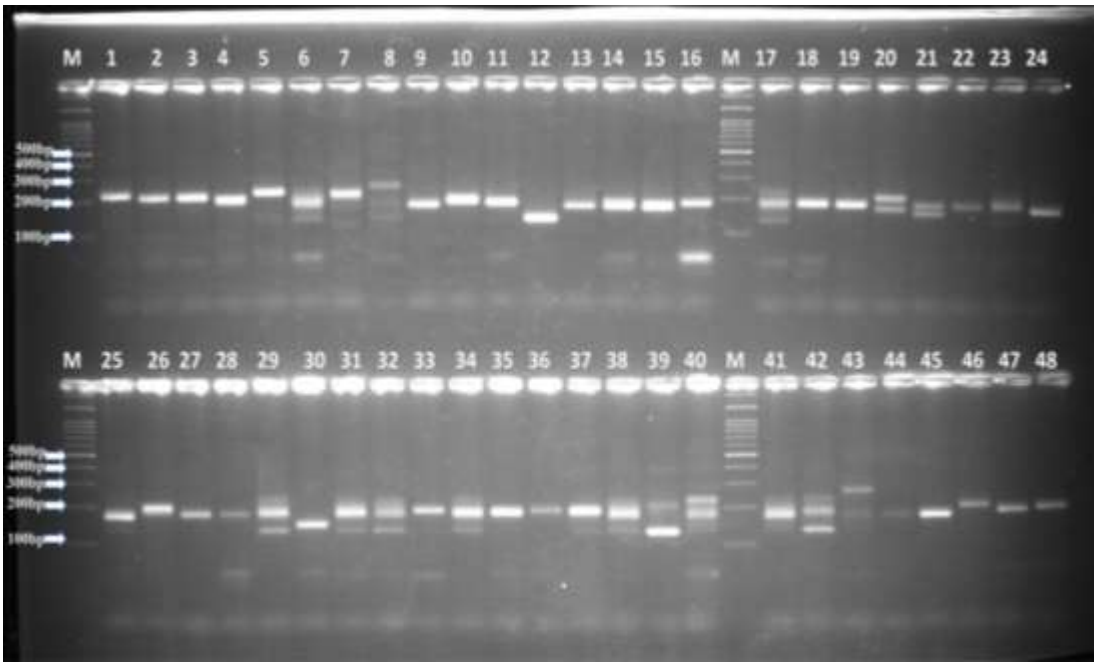
Fig. 4.25: Principal Coordinate Analysis between 3 sub-populations

4.18 Association studies

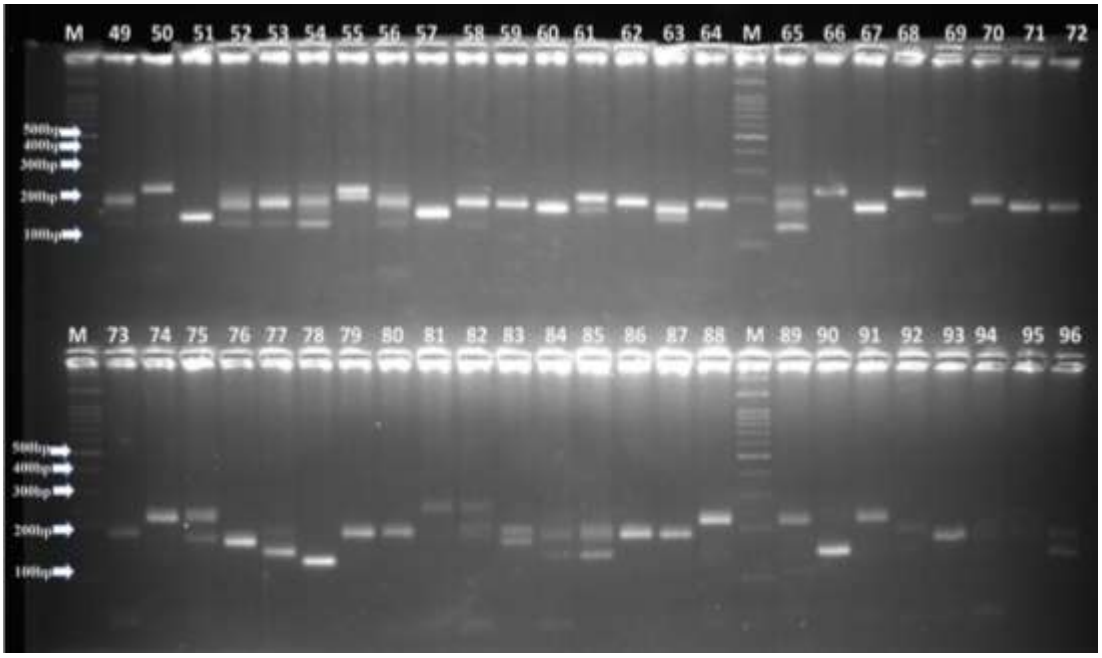
To establish the marker trait relationship, selected genotypes were genotyped using 20 different SSR markers belonging to different chromosomes. Phenotypic data were collected from the lines sown for scrrening against waterlogging and relationship was established using TASSEL (Trait Analysis by Association, Evolution and Linkage) v5 software (Bradbury *et al* 2007) and rMVP R package (Yin *et al* 2021). DNA extracted using the standard protocols were genotyped using SSR markers and scoring was done based on the band polymorphism from the images obtained from Gel Documentation System (Plate 4.11).

4.18.1 Linkage Disequilibrium

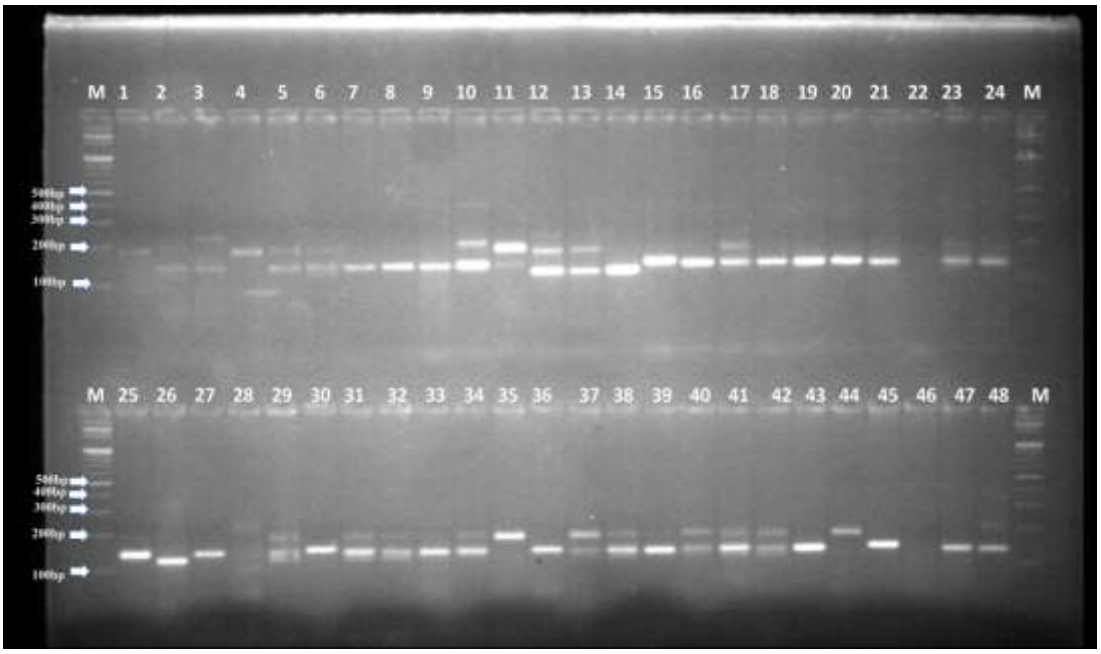
Varieties of software packages are available to conduct the marker-trait association and TASSEL (Trait Analysis by Association, Evolution and Linkage) is the most widely preferred package for conducting such association studies. In our current study we performed the analysis using TASSEL v5 to find the significant association between the phenotypic data



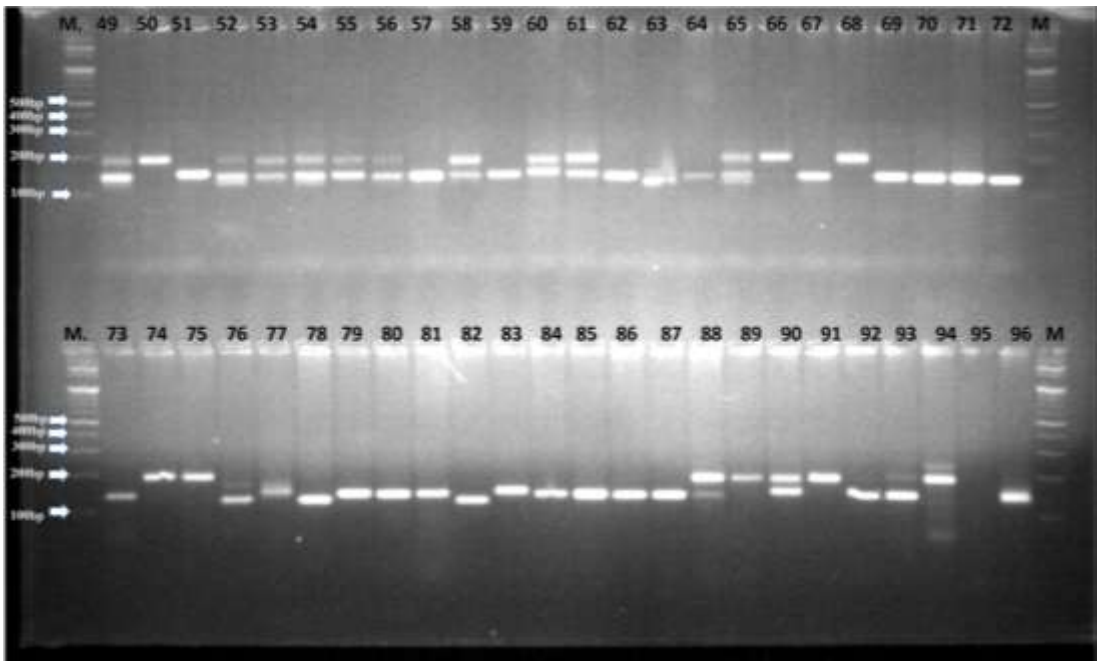
Gel image showing amplicons in 1-48 genotypes when genotyped with bnlg244 marker



Gel image showing amplicons in 49-96 genotypes when genotyped with bnlg244 marker



Gel image showing amplicons in 1-48 genotypes when genotyped with umc2048 marker



Gel image showing amplicons in 49-96 genotypes when genotyped with umc2048 marker)

Plate 4.11: Gel images obtained from genotyping of 96 genotypes using SSR markers

and genotypic data obtained from 20 polymorphic SSR markers belonging to different bins of 5 chromosomes (1, 3, 7, 8 and 9). TASSEL implements two approaches to find the significant association, namely general linear model (GLM) and mixed linear model (MLM) and the result can be visualized in different graphics such as Manhattan plot, Q-Q plot. With the help of TASSEL, linkage disequilibrium (LD) statistics can be worked out and the results can be graphically visualized. In our study we prepare a HapMap file format for our genotyping data and extend of LD was calculated among pairs of alleles. LD thus generated by the TASSEL v5 was represented by disequilibrium matrix displaying r^2 value above the diagonal line and p-value below the diagonal. Seventy seven loci obtained from genotyping using 20 SSR were used for LD statistics and graphically represented in Fig. 4.26.

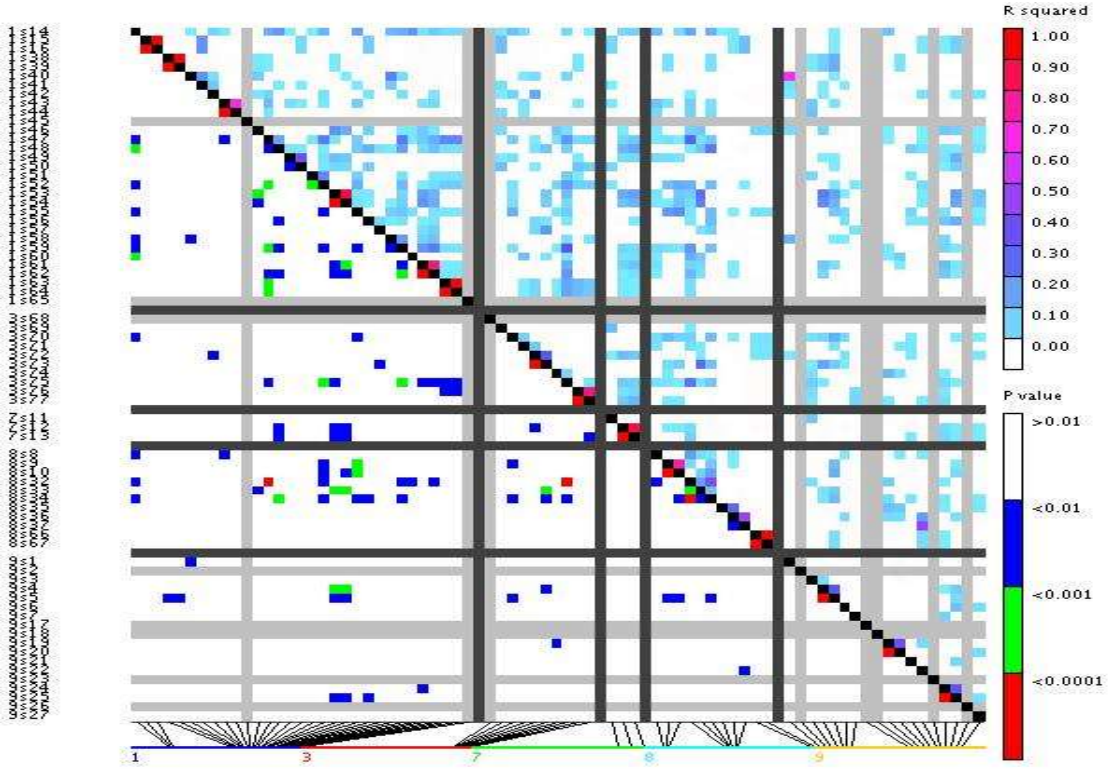


Fig. 4.26: Linkage disequilibrium matrix displaying r^2 above the diagonal and the corresponding p-values below the diagonal. Each cell represent the comparison of two pairs of marker sites with the colour codes for the presence of significant LD. Coloured bar code for the significance threshold levels in both diagonals is shown on right side of the figure

4.18.2 Association study between different traits and markers

Association study was carried out for 54 maize inbred lines with 77 loci generated from 20 polymorphic SSR markers. Each loci were denoted as name of the marker followed by their respective band position in agarose gel. Association results were obtained from the TASSEL v5 package using both GLM and MLM approaches and the data were graphically

illustrated using rMVP package available in R. GLM model incorporates only the principal components to yield the result while the MLM approach incorporates kinship matrix (K) along with population structure data. Data with $-\log_{10}p$ value greater than 3 were presented in the Table 4.38 and 4.39. For MLM approach, the population structure and kinship data were fed to the software and results were presented in table. The population structure for 54 genotypes used for the association mapping is shown in Fig. 4.27. STRUCTURE analysis showed the maximum DeltaK value for our population was obtained at k = 3 indicating three sub-populations present within the population.

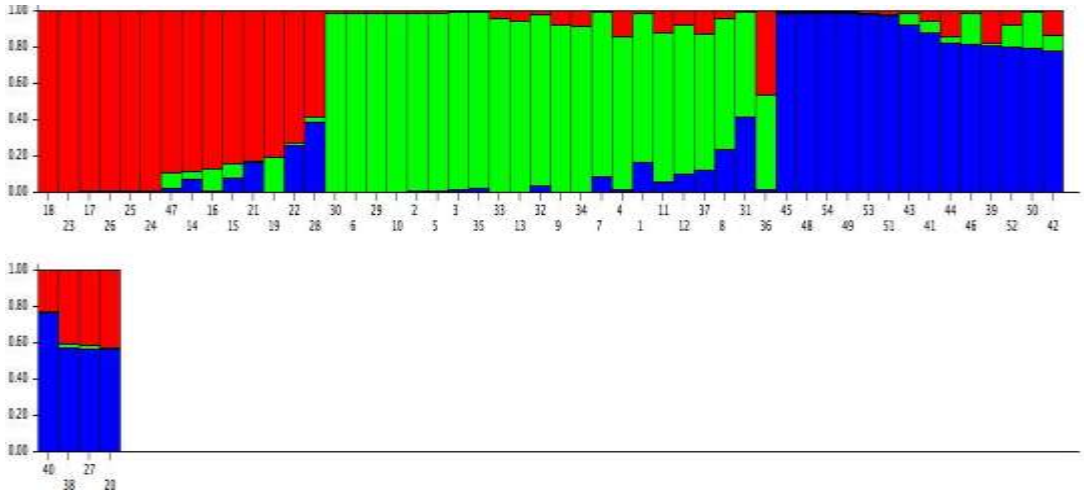


Fig. 4.27: Population structure analysis of 54 genotypes and bar graph showing three sub-populations with best DeltaK at k=3.

4.18.2.1 Association study for root area (RoA):

Three loci; umc1021-70 (chromosome 1), umc2340-650 and umc2340-300 (chromosome 9) showed significant association for root area by both the approaches. Results obtained were presented in table and represented by Q-Q plot and Manhattan plot (Table 4.38) and shown in Fig. 4.28.

4.18.2.2 Association study for root volume (RoV):

Three loci umc1021-70 (chromosome 1), umc2340-650 and umc2340-300 (chromosome 9) showed significant association with the root volume. $-\log_{10}p$ value of 11.34 as per GLM approach and 5.87 as per MLM showed a very high association of these loci to root volume (Table 4.39) and shown in Fig. 4.28.

Table 4.38: Association analysis performed with TASSEL v5 using GLM and MLM models for root area (RoA)

GLM model						MLM model					
Trait	**Marker**	**Chr**	**p**	**-log$_{10}$P**	**R^2**	**Trait**	**Marker**	**Chr**	**p**	**-log$_{10}$p**	**R^2**
RoA	umc1021-70	1	1.22E-04	3.91	0.24646	RoA	umc1021-70	1	9.94E-04	3.00	0.2286
RoA	umc2340-650	9	1.22E-04	3.91	0.24646	RoA	umc2340-650	9	9.94E-04	3.00	0.2286
RoA	umc2340-300	9	1.22E-04	3.91	0.24646	RoA	umc2340-300	9	9.94E-04	3.00	0.2286
RoA	phi001-140	1	0.04088	1.39	0.07794	RoA	phi001-90	1	0.059	1.22	0.0691
RoA	umc1383-220	1	0.06203	1.21	0.0654	RoA	umc1033-130	9	0.07	1.12	0.0609
RoA	umc1383-200	1	0.06203	1.21	0.0654	RoA	umc1052-100	3	0.089	1.05	0.0560
RoA	bnlg1016-140	1	0.07183	1.14	0.06102	RoA	umc2048-150	3	0.095	1.02	0.0536

Table 4.39: Association analysis performed with TASSEL v5 using GLM and MLM models for root volume (RoV)

GLM model						MLM model					
Trait	**Marker**	**Chr**	**p**	**-log$_{10}$P**	**R^2**	**Trait**	**Marker**	**Chr**	**p**	**-log$_{10}$p**	**R^2**
RoV	umc1021-70	1	4.58E-12	11.34	0.57262	RoV	umc1021-70	1	1.34E-06	5.87	0.59611
RoV	umc2340-650	9	4.58E-12	11.34	0.57262	RoV	umc2340-650	9	1.34E-06	5.87	0.59611
RoV	umc2340-300	9	4.58E-12	11.34	0.57262	RoV	umc2340-300	9	1.34E-06	5.87	0.59611
RoV	umc2048-150	3	0.01806	1.74	0.10096	RoV	bnlg182-300	1	0.02874	1.54	0.09986
RoV	bnlg182-300	1	0.02257	1.65	0.09432	RoV	umc2199-100	8	0.03325	1.48	0.09439
RoV	umc2199-100	8	0.02951	1.53	0.08634						
RoV	umc1383-220	1	0.03502	1.46	0.08124						
RoV	umc1383-200	1	0.03502	1.46	0.08124						
RoV	umc2199-120	8	0.04539	1.34	0.07354						
RoV	umc2134-140	9	0.05065	1.30	0.07029						
RoV	bnlg182-400	1	0.06881	1.16	0.06126						

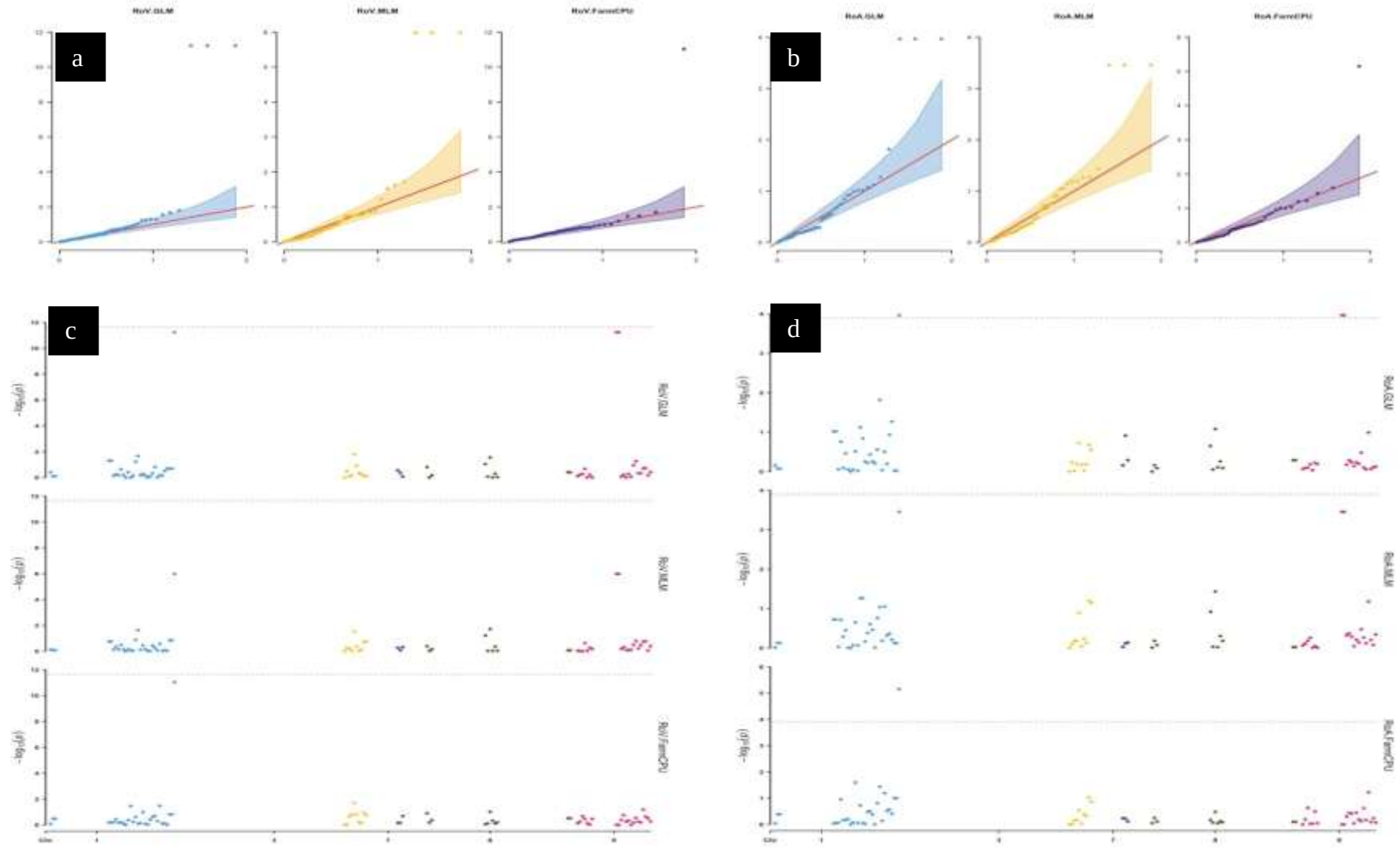


Fig. 4.28: Q-Q plots obtained from association studies using GLM, MLM and FARMCPU for (a) Root volume (RoV) (b) Root area (RoA) Manhattan plots obtained from association studies using GLM, MLM and FARMCPU for (c) Root volume (RoV) (d) Root area (RoA)

CHAPTER-V

SUMMARY

Assigned as Queen of cereals due to its greatest genetic yield potential, Maize is an important food grain crop supplementing a major portion of carbohydrate requirement of the people. Ranked second in terms of acreage, maize is cultivated in almost every part of the world across the several edaphic and growth environments making the crop susceptible to wide range of biotic and abiotic stresses. Among several abiotic stresses, waterlogging is an important abiotic stress just after drought causing a substantial yield loss and depending on the several factors like crop, duration and growth environment, the yield penalty may vary from 15 to 80%. India faces a yield loss of 25-30% each year due to the excessive soil moisture (ESM) led by continuous rainfall and poor drainage creating a hypoxic adverse growth environment, and mineral nutrient and microelements toxicity. In response to the waterlogging, the maize undergoes several morphological, physiological, biochemical and molecular modifications to cope up with the stressful condition to ensure its survival. In Punjab, maize is cultivated in the month of June coinciding with heavy downpour leading to poor germination, plant growth and ultimately causing a yield penalty. Though this stress has been a severe problem for the farmers for a long time, proper study on identification and characterization of maize tolerant lines and allelic variation for the identified genes were lacking and proper study has not been done for this agro-climatic region. Therefore, a study was framed with objectives to screen and identify the tolerant lines from the available germplasms, look for the allelic variation present within the germplasms and to identify the ortholog genes in maize for earlier known genes that have been well characterized for waterlogging and submergence in different related species.

A total of 119 genotypes belonging to different heterotic pools along with one accession of teosinte "*Z mays spp parviglumis*" were procured from the Department of Plant Breeding and Genetics, PAU. These lines were subjected to pre-emergence waterlogging screening which revealed six days of continuous waterlogging is the threshold duration for screening and any longer duration would lead to susceptibility reaction for all the genotypes. These 119 lines showing good germination at control were taken for seedling stage screening. V_2 stage, characterized by two leaves with leaf sheath, has been reported to be the most suitable stage for early seedling stage screening, hence was selected for our experiment. An interval of 3, 6 and 9 days of waterlogging was provided to the seedlings at V_2 stage and percent mortality was recorded to find the lines being most susceptible and the most tolerant. Some lines showed 100% survival which were deemed as most tolerant, while some showed 100% mortality and hence were highly susceptible. With mortality percentage of below 25%,

68 genotypes were highly tolerant, 18 genotypes with mortality between 25-50% were moderately tolerant, 11 genotypes were moderately susceptible with mortality percentage of 50-75% and 23 genotypes with 75-100% mortality were highly susceptible. Though no significant association was found between percentage germination at 6 days of pre-emergence waterlogging and survival at 9 days of waterlogging, few lines showed good results in both the conditions. These includes EML 240, EML 53, I 169, I 172, I 204, LM 16, LM 6, PML 1228, PML 387 and SE 616. Results obtained from phenotypic data and their analysis based on selection indices and multi trait stability index revealed some of the most tolerant lines which include I 172, EML 153, SE 503, EML 125, AVL13119, VL 1010764, I 168, I 204, AVL13056 and PML 368. These lines were highly tolerant and showed the stability across wide interval of waterlogging.

Waterlogging tolerance coefficient (WTC) determination revealed that tolerant lines have the value near to 1 and above while the susceptible lines have lower value. Some lines with high WTC includes EMl 125, EML 124, EML 53, EML 57, I 168, I 172, I 204, PML 373, PML 387. SE 503 and *Z. mays spp parviglumis*. Upon waterlogging all the lines showed declined phenotypic values like root area, root volume, shoot area, dry mass and shoot length as compared with control, but the decline in susceptible lines were much greater than tolerant lines. Similarly, the injury led upon removal of moisture and re-oxygenation was higher in susceptible lines than in tolerant lines. The chlorophyll content as recorded by SPAD meter exhibited that all the lines showed decline in chlorophyll content but the susceptible lines showed sharp decline even in 3 days of waterlogging while the tolerant lines had almost similar level of chlorophyll at 3 days of waterlogging and subsequent decline recorded in 6 and 9 days. Genotypes showing severe reduction (>60%) in SPAD reading at 9 days of waterlogging were EML 279, EML 52, CHT119075, I 200, I 206, I 162, LM 12, LM 20, LM 5, PML 1229, PML 1241, PML 169 and PML 898. Some lines showed tolerance and exhibited minimal reduction in SPAD value (<20%) even at 9 days of waterlogging. These lines were EML 285, EML 124, EML 125, AVL13119, I 172, and *Z. mays spp parviglumis*.

The anthesis silking interval (ASI) was found to be largely affected by waterlogging. Upon 9 days of waterlogging, tolerant lines were able to maintain normal ASI of 2-4 days while in susceptible lines it was as high as 8 days for some highly susceptible lines. Normal ASI (≤ 4 days) even at 9 days of waterlogging were shown by EML 124, EML 125, I 168, I 169A, I 170, I 172, I 192, JCY 20, LM 16, LM 17, LM 26, PML 1228, PML 243, PML 387, SE 503 and SE 616.

Study on aerenchyma tissues revealed that aerenchyma plays a vital role in conferring the tolerance in maize against waterlogging. Root section study showed that percentage

aerenchyma formation was high for tolerant line than for susceptible lines. It was as high as 31.17% for *Z. mays spp parviglumis* and 19.88% for PML 243 but for susceptible line it was 7.86% for I 183. Aerenchyma formation for tolerant lines initiated even at the 10mm from the root tip which was absent for susceptible lines.

Visual observation for barrier against radial oxygen loss (ROL) showed that barrier formation for the avoidance of leaching out of the air across the length of the root was higher for tolerant lines while no barrier formation or minimum barrier formation was found for susceptible lines. Lines AVL13119, I 168, I 172, I 185, LM 16, PML 28 and *Z. mays spp parviglumis* had barrier formation of more than 90% while lines I 171, I 205, I 206, LM 12, I 189 showed completely blue colouration indicating no barrier formation at all.

For orthologous gene identification two well-characterized submergence tolerance genes of rice *viz., Sub1A* and *Snorkel* were selected and orthologs were searched using the BLAST NCBI. Results from the BLAST study showed no significant similarity for *SNORKEL* gene while *Sub1A* gene showed significant sequence similarity for 2 out of 3 coding sequences (CDS), CDS1 and CDS3 with sequence similarity of 78.46% and 16% respectively which were used to design gene specific primers and used for identification of allelic variation in our lines. Genotyping with these markers showed no polymorphism and showed non-specific binding for the population and hence to have a clear understanding for these genes gene expression analysis was opted. For gene expression study, RNA was isolated from root tissues at different duration of waterlogging and gene specific primers were used for RT-PCR using 18s as an internal control. Gene expression study revealed that the expression of both *Sub1 CDS1* and *Sub1 CDS3* orthologs were higher in the tolerant lines (I 172 and I 168) as compared to susceptible lines (I 200 and PML 898) during early duration of waterlogging. The expression for *Sub1A CDS1* ortholog was 12.53 and 7.38 folds for I 172 and I 168 respectively, while the same was 2.85 and 0.81 folds for I 200 and PML 898 respectively. Similarly for *Sub1A CDS3* ortholog it was 15.85 and 2.23 for I 172 and I 168 respectively and 0.64 and 0.72 for I 200 and PML 898 respectively. But the expression declined in 6 and 9 days suggesting that both these genes might be responsible for adjusting the plants against abrupt growth environment and helping plants to adapt themselves to cope up against waterlogging in early period of waterlogging and as the duration of flooding tend to increase their expression falls down to normal. *Z. mays spp parviglumis* on the other hand had the fold change of 1.93 and 1.72 for *Sub1A CDS1* and *Sub1A CDS3* orthologs respectively suggesting that these orthologs did not play any significant role in conferring tolerance to them as even though the expression of gene was low the plants were exhibiting highest degree of tolerance.

Population structure analysis revealed that there are three sub-populations in our available lines and AMOVA study showed that the percentage variation among population is 9%, among individuals is 37% and within individuals is 55%.

Twenty SSR primers were used to identify the allelic variation present in the germplasm and to establish the marker trait relationship. These markers showed polymorphism across the lines and 83 allelic loci were detected with an average of 4.15 loci per marker. The PIC ranged from 0.2289 for umc2210 to 0.7530 for bnlg244 with an average of 0.503. Major allele frequency ranged from 0.8593 (bnlg1812) to 0.302 (bnlg2440) with an average value of 0.557. The heterozygosity varied from 0 (bnlg2199, umc1383, umc2210) to 0.9896 (umc1021) with an average of 0.3367.

Marker trait association was established using the data obtained from SSR genotyping and phenotypic data collected from the seedlings using TASSEL v5 software. Both GLM and MLM model were employed to find the association. Two markers; umc1021 and umc2340 demonstrated significant association with root area ($-\log_{10}$p value = 3.91) and root volume ($-\log_{10}$p value = 11.34). Other phenotypic traits showed the $-\log_{10}$p value of 2.5 and lower showing insignificant association. Results were graphically presented using Manhattan and Q-Q plot obtained from rMVP R package. Manhattan plot showed significant association using GLM, MLM and FARMCPU model.

A subset of eighty maize inbred lines from experimental materials were observed for 28 DUS parameters recording several morphological observations. Out of 28 DUS parameters, 20 were visually assessed parameters and data were based on visual observation while the 8 parameters werer measurable DUS characters. Separate clustering analysis based on both DUS parameters grouped the lines into two major clusters. Correlation study among several measurable DUS characters revealed negative correlation between cob length and cob diameter (-0.25), positive correlation between cob length and tassel length (0.32) and plant height (0.41). Tassel length and plant height showed positive correlation (0.26). Cob length and leaf blade width also showed positive correlation (0.34). A positive correlation between cob diameter and 1000 kernel weight (0.25) and between silking and anthesis days (0.54) were also observed.

In the current study, several morphological and molecular aspects of waterlogging were studied and based on the results several lines were deemed as tolerant or susceptible. Allelic variation was studied and association study showed some association between the markers and our phenotypic traits. Submergence tolerance gene's orthologs in maize is found to have some relation in conferring tolerance against waterlogging stress.

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APPENDIX - I

List of DUS characters, expression, score, stage and type of assessment

S. No.	Characteristics	Expression	Score	Stage of observation	Type of assessment	Instrument
1.	Leaf: Angle between blade and stem (on leaf just above upper ear)	Narrow (<45^o)	1	After anthesis has begun	VG	Visual
		Wide (>45^o)	9			
2.	Leaf: Attitude of blade (on leaf just above upper ear	Straight	1	After anthesis has begun	VG	Visual
		Droopy	9			
3.	Stem: Anthocyanin of blade (on leaf just above upper ear)	Absent	1	50% of anthesis	VS	Visual
		Present	9			
4.	Tassel: Time of anthesis (on middle third of main axis,50% of plants)	Very early (<45 days)	1	50% of anthesis	VG	Visual
		Early (45-50 days)	3			
		Medium (50-55 days)	5			
		Late (>55 days)	7			
5.	Tassel: Anthocyanin colouration at base of glume (in middle third of main axis)	Absent	1	50% of anthesis	VS	Visual
		Present	9			
6.	Tassel: Anthocyanin colouration of glume excluding base(in middle third of main axis)	Absent	1	50% of anthesis	VS	Visual
		Present	9			
7.	Tassel: Anthocyanin colouration of anthers (in middle third of main axis on fresh anther)	Absent	1	50% of anthesis	VG	Visual
		Present	9			
8.	Tassel : Density of spikelet (in middle third of main axis)	Sparse	3	50% of anthesis	VG	Visual
		Dense	7			
9.	Tassel: Angle between main axis and lateral branches (in lower third of tassel)	Normal (<45^o)	1	50% of anthesis	VG	Visual
		Wide (>45^o)	9			

S. No.	Characteristics	Expression	Score	Stage of observation	Type of assessment	Instrument
10.	Tassel: Altitude of lateral branches (in middle third of tassel)	Straight	1	50% of anthesis	VG	Visual
		Curved	5			
		Strongly curved	9			
11.	Ear: Time of silk emergence (50% plants)	Very early (<48 days)	1	50% of anthesis	VG	Visual
		Early (48-53 days)	3			
		Medium (53-58 days)	5			
		Late (>58 days)	7			
12.	Ear: Anthocyanin colouration of silks (on day of emergence)	Absent	1	50% of anthesis	VG	Visual
		Present	9			
13.	Leaf : Anthocyanin colouration of sheath (below the ear)	Absent	1	Caryopsis at watery ripe	VS	Visual
		Present	9			
14.	Tassel :Length of main axis above lowest side branch	Short (<20 cm)	3	Caryopsis at watery ripe	MS	30 cm Scale
		Medium (20-30 cm)	5			
		Long (>30cm)	7			
15.	Inbred line only	Short (<120 cm)	3	Medium milk	MS	2m scale
		Medium (120-150 cm)	5			
		Long (>150 cm)	7			
16.	Plant: Ear placement	Low	3	Medium milk	MS	2m scale
		Medium	5			
		High	7			
17.	Leaf :Width of blade (leaf of upper ear)	Narrow (<8 cm)	3	Medium milk	MS	30 cm scale
		Medium (8-9cm)	5			
		Broad (>9 cm)	7			

S. No.	Characteristics	Expression	Score	Stage of observation	Type of assessment	Instrument
18.	Ear: Length without husk	Short (<10 cm)	3	Dehusking	MS	Vernier caliper
		Medium (10-15 cm)	5			
		Long (>15 cm)	7			
19.	Ear: Diameter without husk (in middle)	Small (<4 cm)	3	Dehusking	MS	Vernier caliper
		Medium (4-5 cm)	5			
		Large (> 5 cm)	7			
20.	Ear: Shape	Conical	1	Dehusking	VG	Visual
		Conical-Cylindrical	2			
		Cylindrical	3			
21.	Ear: Number of rows of grains	Few (≤ 8)	3	Dehusking	MS	Visual
		Medium (10-12)	5			
		Large ((≥ 14)	7			
22.	Ear: Type of grain (in middle third of ear)	Flint	1	Dehusking	VG	Visual
		Semi flint/semi dent	2			
		Dent	3			
23.	Ear: Colour of top of grain	White	1	Dehusking	VG	Visual
		White with cap	2			
		Yellow	3			
		Yellow with cap	4			
		Orange	5			
		Red	6			
		Variegated	7			

S. No.	Characteristics	Expression	Score	Stage of observation	Type of assessment	Instrument
24.	Ear: Anthocyanin coloration of glumes of cob	White	1	Threshing	VG	Visual
		Light purple	2			
		Dark purple	3			
25.	Kernel: Row arrangement (middle of ear)	Straight	1	Threshing	VG	Visual
		Spiral	2			
		Irregular	3			
26.	Ear : Type of Grain	Field corn	1	Threshing	VG	Visual
		Pop corn	2			
		Waxy/opaque	3			
		Opaque tinge	4			
		Sweet corn	5			
27.	Kernel shape	Shrunken	1	Threshing	VG	Visual
		Round	2			
		Indented	3			
		Toothed	4			
		Pointed	5			
28.	Kernel size (1000 kernel weight)	Very small (<100g)	1	Threshing	MG	Weighing balance
		Small (100-200g)	3			
		Medium (200-300g)	5			
		Large (>300g)	7			

VITA

Name of the student	**:**	Sittal Thapa
Father's name	**:**	Mr. Ujir Singh Thapa
Mother's name	**:**	Mrs. Kanchhi Thapa
Nationality	**:**	Nepalese
Date of birth	**:**	28.06.1994
Permanent home address	**:**	Ratnanagar 12, Jamunapur, Chitwan, Bagmati Province, Nepal
e-mail address	**:**	ctalthp@gmail.com

EDUCATIONAL QUALIFICATION

Bachelor Degree	**:**	B.Sc. (Agriculture)
University and year of award	**:**	Banaras Hindu University, Varanasi 2019
OCPA	**:**	8.99/10.00
Master's Degree	**:**	M.Sc. (Plant Breeding and Genetics)
University and year of award	**:**	Punjab Agricultural University, Ludhiana 2021
OCPA	**:**	8.87/10.00
Title of Master's Thesis	**:**	Identification of maize genotypes tolerant to waterlogging and their characterization using rice orthologs
Awards/Distinctions/Scholarships/ Fellowships	**:**	▪ Champa Devi Gold medal during B.Sc. for second highest OCPA ▪ Study in India (SII) scholarship by Govt. of India