DISSERTATION REPORT
(AGR- 596)

"AMELIORATIVE EFFECT OF PUTRESCINE AND MYCORRHIZA ON WATERLOGGED STRESS IN MAIZE"

Synopsis Submitted To

Lovely Professional University, Punjab

In Partial Fulfilment of the Requirements for the
Degree of

Master of Science (Agriculture)
In
Agronomy
By

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November 2017

CERTIFICATE

I certified that this synopsis **Johnson Yumnam** with registration no: 11718431, **TOPIC- AMELIORATIVE EFFECT OF PUTRESCINE AND MYCORRHIZA ON WATERLOGGED STRESS IN MAIZE.**

” has been formulated and finalized by the student on the subject.

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(Signature of Supervisor)

Dr. Prasant kumar
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DECLARATION

I hereby declare that the project work entitled " **TOPIC- AMELIORATIVE EFFECT OF PUTRESCINE AND MYCORRHIZA ON WATERLOGGED STRESS IN MAIZE.**

" is an authentic record of my work carried out at lovely professional university as requirements of project work for the award of degree of Master of Science in Agronomy, under the guidance of Dr. Prasant kumar, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India.

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CONTENT

INTRODUCTION

Maize (*Zea mays* L.) is known as the queen of cereals because of its higher genetic yield potential among the cereals. Its probable place of origin lies in Tehuacan valley of Mexico (Lowa St. Univ., 2014).Its chromosome number is 2n=20 and belongs to the family Poaceae.It is a most versatile emerging crop having wider adaptability to varied agro-climatic conditions.It is grown from 58^{o}N to 40^{o}S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year and with a growing cycle ranging from 3 to 13 months (CIMMYT 2000).It is a third important cereal with highest production and productivity next to wheat and paddy. It is a well known fact that maize is a heavy feeder for both nutrients and soil moisture as well as also is a high yielding, easy to process, readily digestible and cheaper than other cereals. However the major maize production areas are located in temperate regions of the globe. The United States, China, Brazil and Mexico account for 70% of global production. India has 5% of corn acreage and contributes 2% of world production. In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed (AICRP on Maize, 2007). In the last one decade, it has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirement (Andersene and Kupper H. 2013). Maize is an important multipurpose cereal crop used as food, feed, fodder, fuel and in the manufacture of industrial product. Generally, maize kernels are used either for food or feed for livestock (Anda, 2011, Ardnini 2004). The grains are rich in vitamin viz., A, C and E, carbohydrates, essential minerals and protein viz., zein, which is a class of prolamine protein. In addition to staple food for human being and quality feed for animals, maize serves as a basic raw material and as an ingredient to thousands of industrial products that includes starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package and paper industries etc. Maize is grown in about 166 countries occupying 165 m ha area with the production of more than 800 m t and a productivity of 5.1 t ha^{-1}. In India maize has higher growth rate among food crops contributing 5 percent area, 2.4 percent production to world maize. Maize production in India was around 23 Million tonnes in 2013-14. The area under maize cultivation was around 9.4 Million hectare in 2013-14, which accounts nearly 9 per cent to the national food basket and more than 155 billion to the agricultural GDP (Azizian et al., 2013)

. A polyamine is an organic compound having two or more primary amino groups-NH2. They are also known as a group of natural compounds with an aliphatic nitrogen structure present in almost all living organisms. It plays an important role in many physiological processes such as cell growth and development. Polyamines which are commonly found are putrescine. Spermidine, spermine etc. the diamine putrescine, the triamine spermidine and the tetramine spermine are ubiquitously found in plant cells while other polyamines are of more limited occurrence. In animals, their levels are maintained from both the diet and denovo synthesis. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase. Polyamine can also be synthesized from the aminoacid arginine and methionine. The first step in the pathway is the production of ornithine from arginine by the mitochondrial enzyme arginase. Ornithine is then decarboxylated by ornithine decarboxylase to produce putrescine. Polyamine is found in high concentration in the mammalian brain. The polyamines declines with the ages in the organism.

Mycorrhiza is a fungus having a symbiotic or mutualistic relationship with the rhizosphere or roots of the plants. They share a mutualistic relationship in which the mycorrhiza forms a network of filaments that associates with the plant roots which helps them in uptaking the mineral nutrients or water while the mycorrhiza are benefitted by getting access to carbohydrates such as glucose and sucrose. The carbohydrates are translocated from their source to root tissues and onto the plant fungal partners. Some plant roots may be unable to uptake nutrients that are chemically or physically immobilized. For example, phosphate ions and micronutrients such as ions. Polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes (Farooq *et al*., 2009). They are the recent additions to the class of plant growth regulators, and also considered as a secondary messenger in signaling pathways (Kusano *et al*., 2008). Polyamines are involved in abiotic stress tolerance in plants (Nayyer *et al*., 2005). Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss, and stimulation of protein, nucleic acid and protective alkaloids (Sharma, 1999). Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions (Roberts *et al*., 1986). Polyamines also protect membranes from oxidative damages as they as free radical scavengers (Besford *et al*., 1993).

HYPOTHESIS

For the present study, I select maize plant, because

[A] The research of the waterlogged effect of on the maize plant is limited.

[B] Maize which is often as the feed sources and tolerance for waterlogged stress is not known that’s why evidence of presence of tolerance in maize is still a point of research;

OBJECTIVE

The objectives of my work are to study:

1. The effect of waterlogged stress on various morphological, biochemical and yield attributes in maize.

2. The effect of polyamines (putrescine) and mycorrhiza in ameliorating waterlogged induced stress in maize.

REVIEW OF LITERATURE

Zaidi et al (2005) studied that the Excess moisture during the summer-rainy season was one of the production constraints for maize. remarkable variability was found among the genotypes studied. Genotypes with good carbohydrate accumulation in stem tissues, moderate stomatal conductance. <5 days ASI, high root porosity, and early brace root development ability have been found to have good tolerance against the hypoxia/anoxia caused by excess soil moisture conditions.

Ren et al (2013) field experiment was performed to study the effects of waterlogging for different durations (3 and 6 d) on the yield and growth of summer maize at the three-leaf stage (V3), six leaf stage (V6), and the 10th day after the tasseling stage (10VT). The results after 2 year indicated that maize development and grain yield responses to waterlogging depended on both stress severity (intensity and duration) and different growth stage. Yield decreased significantly with an increased waterlogging duration during V3 and V6.

SAIRAM et al. (2008) studied that the main cause of damage under waterlogging was oxygen deprivation, which affect nutrient and water uptake, so the plants show wilting even when surrounded by excess of water. Lack of oxygen shift the energy metabolism from aerobic mode to anaerobic mode. Besides, non-symbiotichaemoglobins and nitric oxide have also been suggested as an alternative to fermentation for maintaining lower redox potential (low NADH/NAD ratio), and thereby playing an important role in anaerobic stress tolerance and signalling.

Abiko et al.(2012) Enhancement of oxygen transport from shoot to root tip by the formation of aerenchyma and also a barrier to radial oxygen loss (ROL) in roots is common in waterlogging-tolerant plants. *Zea nicaraguensis* (teosinte), a wild relative of maize (*Zea mays* ssp. *mays*), grows in waterlogged soils. The relationships between the ROL barrier formation and suberin and lignin depositions in roots are discussed. The ROL barrier, in addition to aerenchyma, would contribute to the waterlogging tolerance of *Z. nicaraguensis*.

Liu et al.(2010) Waterlogging strongly affects agronomic performance of maize (*Zea mays* L.). In order to investigate the suitable selection criteria of waterflooding tolerant genotypes, and identify the most susceptible stage and the best continuous treatment time to waterlogging, 20 common maize inbred lines were subjected to successive artificial water-flooding at seedling stage, and waterlogging tolerance coefficient (WTC) was used to screen water-flooding tolerant genotypes. he results showed that the second leaf stage (V2) was the most susceptible stage, and 6 d after water-flooding was the best continuous treatment time. Dry weight (DW) of both shoots and roots of all lines were significantly reduced at 6 d time-point of waterlogging, compared to control.

Lennard (2004) This paper reviews a range of studies under controlled conditions (glasshouse and growth cabinet) focusing on the effects of the interaction between waterlogging (hypoxia) and salinity on the ion relations, growth and survival of higher plants. These increased concentrations in the shoots have adverse effects on plant growth and survival. It is argued that the interaction between waterlogging and salinity has major implications for saltland management, and for the selection and breeding of plants adapted to saltland.

Yong-zhong LIU(2009) identify the most susceptible stage and the best
continuous treatment time to waterlogging and reported that 20 common maize inbred lines were subjected to successive artificial water flooding at seedling stage, and waterlogging tolerance coefficient (WTC) was used to screen water flooding tolerant genotypes.

Ren, B, Zhang (2014) has reported that maximum grain-filling rate decreased under water logging, furthermore, the dry matter accumulation decreased and dry matter distribution proportions of the stem and leaf increases. However, the distribution proportion of grain decreased.

Anonymous (1999) has reported that maize can be grown over a diverse environmental and geographical range in comparison to and other cereal crop.

Stanilsaw Grzesiak(1999) has reported that plant grown under waterlogging
showed a smaller number and less dry matter of lateral branching than plant gown in normal or control condition.

Baizhao Ren (2016) has reported that summer maize root growth and grain yield to watterloggin depended on the growth stage and summer maize is most susceptible to waterlogging damage.

Jerald Anthony(2016) has reported that maize with higher total chlorophyll and longer nodal roots has a higher chance of survival and higher yeidl components when subjected to waterlogged condition.

Zurarah Zubairi(2012) has reported that in waterlogged condition leaves present on the lower portion of the plant show leaf senescence and became bronze in colour and concluded that water logging influence the growth and yield of maize plant.

Kono *et al.*, (1987) has reported that in waterlogged condition losses of seminal and nodal roots are smaller in size and it is responsible in reduction the matrix potential.

J.P Shivastava (2006) has reported that genotype which repuire low level of
nitrogen for normal growth, tolerates waterlogging stress and suggested that high level of potassium in resistant genotype is advantageous in maintaining plant water relation and leaf conductance.

Thomson *et al.,(*1989) has reported that water logged alters mineral nutrient status of plant and derangements in mineral nutrints acquisition also affect plant growth and development.

Scholowing and Techming (1997) has reported that water logging is a serious problem for maize and nearly 1.5 m ha land under maize is affected by waterlogging, resulting in an average loss of 25-30% maize production every year.

Setter *et al.*, (1989) has reported that genotypes with high biomass have been reported to sustain better growth and development after release on water logging stress condition.

Grineva *et al.,(*1988) has reported that excess soil moisture reduces leaf area in maize irrespective of genotype.

Kumutha *et al.*, (2008) has reported that reduction in chlorophyll content under excess moisture conditions increased chlorophyll degradation and decrease chlorophyll synthesis.

Rai *et al.*, (2004) has reported that sugar content reduced in a peak level due to waterlogging in maize.

Ferreira *et al.*, (2008) has reported that waterlogging stress induced low oxygen concentration in soil (hypoxia) or complete absence of oxygen (anoxia) which effected the nutrient uptake, synthesis and translocation of growth regulators, photosynthesis, respiration and carbohydrate partitioning and decreased the yieldof maize crops.

METHODOLOGY

The pot experiment will be conducted in the poly house of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one genotype of maize. Maize genotype will be taken from Punjab Agriculture University, Punjab. Pot size for the experiment will be diameter: 30 cm and height 25 cm. Waterlogged stresses will be created in plant by exogenous application of water in soil. Putrescine will be applied at the rate of 1.50 mM and 3.0 mM through foliar application. The various measurements will be made at three stages such as 30 day, 60 days and 90 days.

EXPERIMENTAL DETAILS

1. Genotype: Local Variety

2. No. of treatment

a. Control

b. Waterlogged

c. Waterlogged + Endomycorrhizal fungi (AMF), *Glomus* species

d. Waterlogged + Putrescine 1(1.50 mM)

e. Waterlogged + Putrescine 2 (3.0 mM)

f. Control + Putrescine 1

g. Control + Putrescine 2

h. Control + Endomycorrhizal fungi (AMF), *Glomus* species

i. Waterlogged + Putrescine 1(1.50 mM) + Endomycorrhizal fungi (AMF), Glomus species

j. Control + Putrescine 2 (3.00 mM) + Endomycorrhizal fungi (AMF), *Glomus* species

3. Replication: four.

4. Treatment: 10

5. Total number of pots: 10 x 3= 30

6. Design: CRD

OBSERVATIONS TO BE RECORDED

The various measurements will be made at three stages such as 30 day, 60 days and 90 days.

1. Morpho-physiological parameters

a. Germination percentage (for screening)

b. Plant Height (cm)

c. Leaf area (cm x cm)

d. Leaf number

e. Days to 50% anthesis

f. Days to maturity

g. Internodal length (cm)

h. Stem girth (cm)

2. Biochemical parameters

a. Chlorophyll content (Arnon, 1949)

b. Total soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

c. Total soluble protein (Bradford method, 1976)

d. Lipid peroxidation (Malondialdehyde-MDA) (Hodges *et al*., 1991)

e. Catalase (EC. 1.11.1.6) (Teranish *et al*., 1974)

f. Membrane stability (Sullivan method, 1972)

g. Phenylalanine ammonia lyase (EC. 4.3.1.5) (Brueske, 1980)

h. Superoxide dismutase (SOD) (EC. 1.15.1.1) (Dhindsa *et al*., 1981)

i. Transportable ratio (Using Atomic absorption spectrophotometer in different parts of plant including developing seeds).

3. Anatomical features

Transverse section of root to study mycorrhizal colonization (Microscopic conventional method)

4. Yield and quality attributes

1. Number of grains per cob

2. Number of cob

3. Test weight

4. Seed soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

5. Seed total protein content (Bradford method, 1976)

5. PLAN OF WORK

1st Year

(a) Morpho-physiological parameters

(b) Determination of biochemical parameters

(c) Anatomical features

(d) Yield and quality attributes

2nd Year

The experiment conducted in 1st year will be repeated during the IInd year.

Figure: total soluble sugar.

A) Estimated the total soluble sugar (anthrone method) in seed.

s.no	Blank reading	0.000
1	R1	0.611
2	R2	0.636
3	R3	0.623

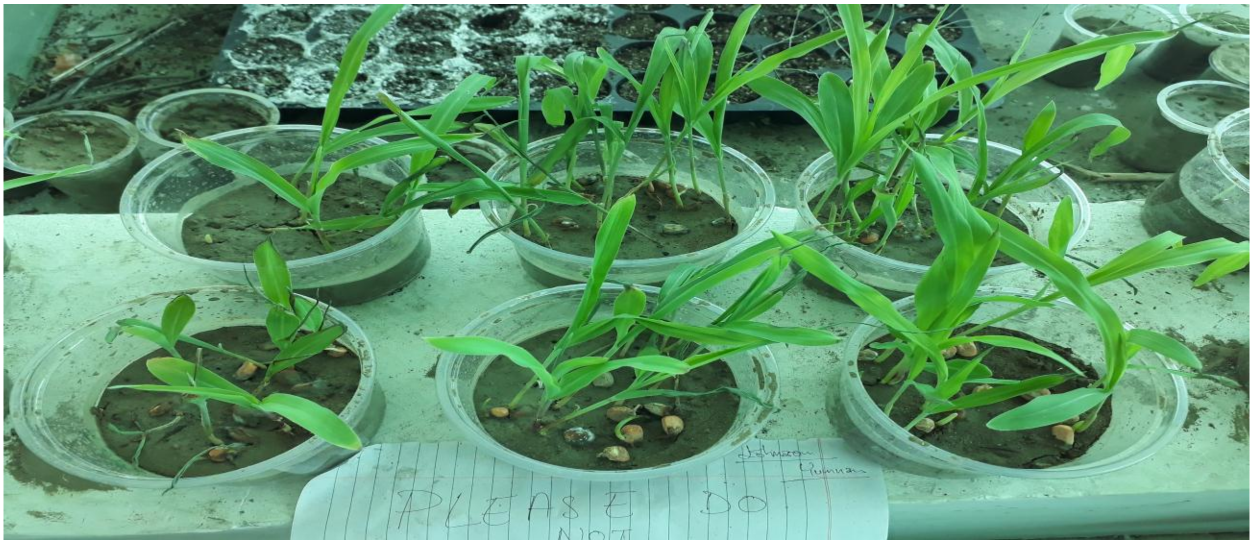
Figure: total soluble protein

.

B) Estimation of total soluble protein. (Bradford method).

s.no	Blank reading	0.000
1	R1	0.326
2	R2	0.621
3	R3	0.419

photo- control



(Source: Photograph by Johnson Yumnam, 2018, unpublished)

Photo-Treatment with mycorrhiza without waterlogged.

Preliminary test.

200 g soil = 2g mycorrhiza, 100ml water

200g soil = 4g mycorrhiza, 100ml water.

200g soil = 6g mycorrhiza, 100ml water.

200g soil = 8g mycorrhiza, 100ml water.

200g soil = 10 mycorrhiza, 100ml water.

CONCLUSION

Based on the observed data, I have found the amount total soluble sugar in maize grain is 0.636 and the amount of total soluble protein is 0.621, respectively. I have treated the plant under water logged stress with putriscine with 0.1mg and mycorrhiza respectively. The plant treated with mycorrhiza was unable to survive in pots because of higher toxicity induced. So far the new setup of the treatment along with mycorrhiza has been stablished with the rate of 2, 4, 6, 8 and 10 gram mycorrhiza in 200g of soil.

ACKNOWLEDGEMENT

I am very thankful to my supervisor Dr.Prasann Kumar for this consistent mral encouragement and support during the experimental work. I am also thankfull to all lab technician for their support . I am highly thankful to department of Agronomy ,school of Agriculture ,Lovely Professional University for providing each and every support during the work.

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