EFFECTS OF ABIOTIC STRESSES ON THE GROWTH OF *Vigna radiata*

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ABSTRACT

Abiotic stresses are discouraging the normal growth and production of crops. Unlike animals, plants which do not have the motile system are unable to escape from this disaster. Hence, this experiment was to determine the effects of abiotic stresses (osmotic stress, salinity and heavy metal stress) on the growth of *Vigna radiata*. In this study, the seeds of *V. radiata* were cultured onto black soil supplemented with Murashige and Skoog (MS) medium which served as control or MS medium augmented with various concentrations of abscisic acid (ABA) (1 mg/L, 2 mg/L, 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L), sodium chloride (NaCl) (1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L and 40 mg/L) or cadmium (Cd) (0.00005 mg/L, 0.00025 mg/L, 0.00050 mg/L, 0.00250 mg/L, 0.01250 mg/L). The number of leaves and stem height of *V. radiata* were observed and recorded over a period of 22 days. Under the treatment of ABA, the leaves turned from green to yellow and eventually withered. The highest mean number of leaves was 2 per explant at 1 mg/L, 2 mg/L, 5 mg/L and 15 mg/L ABA. The highest mean stem height was 21.058 cm at 2 mg/L ABA. On the other hand, NaCl stress resulted in the formation of dried leaves. The peak average number of leaves was 2 per explant at 1 mg/L and 5 mg/L of NaCl while the peak of average stem height was 17.15 cm at 1 mg/L NaCl. Cd showed its effect by turning the greenish leaves into yellowish and the leaves became soft. The optimal mean of amount of leaves was 2 at all concentrations tested while 0.00050 mg/L of Cd yielded the optimal average of stem height, which was 18.85 cm. Leaf chlorosis was also observed in the Cd treatment. In a nutshell, the abiotic stresses (ABA, NaCl, Cd) utilized in this study was proven to impose their negative effects on the growth of *V. radiata*.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius: Unit of Temperature
µM	Micromolarity: Unit of Concentration
ABA	Abscisic acid
ANOVA	Analysis of Variance
Cd	Cadmium
Cl^-	Chlorine ion
Co	Cobalt
Cu	Copper
DNA	Deoxyribonucleic acid
Fe	Iron
g/mL	Gram per millilitre: Unit of concentration
GSH	Glutathione
H^+	Hydrogen ion
HCl	Hydrochloric acid
Hg	Mercury
K^+	Potassium ion
mg	Milligram: Unit of mass
mg/L	Milligram per litre: Unit of concentration
MS	Murashige and Skoog
Na^+	Sodium ion
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate

NaOH	Sodium hydroxide
Ni	Nickel
Pb	Lead
pH	power of hydrogen: Measure of concentration of hydrogen ion
Zn	Zinc

CHAPTER 1

INTRODUCTION

By far, environmental disruption has caused the decreasing yield of crops in 20% in the cultured territory and approximately 50% in all irrigated territory all over the world respectively (Rhoades & Loveday, 1990). Not only for plants, all living organisms throughout the earth require certain functional mechanism to strategize themselves when encounter with various stress sources such as climate, heavy metal pollution, drought and so on. Creatures which are able to move can avoid them but for plants, it is too difficult to do so. Scientists have performed several experiments and found out that a numerous genes are responsible for plant structure, shape and organic chemistry at the genetic level and directly lay impacts for them (Ziaf et al., 2016). Therefore, this leads them to develop a biochemical and morphological evolutions as to sustain the disastrous significances brought by the environment (Petrov, Hille, Mueller-Roeber, & Gechev, 2015).

Nowadays, the attempts contributed by scientists to further the abiotic stress endurance of plants with alleles surviving under the unfavorable conditions have led to creditably remarkable improvements (Khan, Ahmad, & Khan, 2015). However, the current new applications such as *in vitro* mutagenesis, tissue culture and genetic transformation (Dita, Rispail, Prats, Rubiales, & Singh, 2006) must come through various difficulties and shortcomings because of the complicated characteristics of abiotic factor endurance (Khan et al., 2015).

Abiotic stress is a crucial limitation for the final production of crops. In general, it can be known as the unfavorable environmental circumstances such as salinity, nutrient stress and alteration of temperature which will damage the morphological and physiological developments within plants (Atkinson & Urwin, 2012) while in comparison, biotic stress is often referred as the damages to plants given by the living organisms such as microbes and herbivores (Rejeb, Pastor,

& Mauch-Mani, 2014). A typically negative example for abiotic influence is the total production of crop. The climatic alteration and the water sources provide certain pressure towards the growth and yield of crop plants, and thus indirectly affects the food productivity globally (Atkinson & Urwin, 2012). However, for plant phenotypic comebacks when treating with abiotic stress, there is only little information found (Pandey, Ramegowda, & Senthil-Kumar, 2015).

Owing to the expeditious urbanization and industrialization among nations, heavy metal pollution has become a dreadful problem (Jiang et al., 2013). The contaminated soil with heavy metals shows harmfulness to human in many ways, such as inhalation, skin contact and food sources (Jiao, Teng, Zhan, Wu, & Lin, 2015). Also, there is possibility that heavy metal can pollute the deep groundwater. The hazardous cadmium (Cd) metal and its compounds basically emanated from rechargeable nickel–cadmium batteries. Bone and kidney functions of human will be damaged with the contact of Cd (Jarup, 2003).

Therefore, in this experiment, the propagation of the *Vigna radiata* was carried out to determine the effect of abiotic factors, namely abscisic acid (ABA), sodium chloride (NaCl) and Cd on the growth of *V. radiata*.

CHAPTER 2

LITERATURE REVIEW

2.1 *Vigna radiata*

2.1.1 Characteristics of *V. radiata*

V. radiata L, common name known as mung bean, is found to be one of the most focal legumes in both tropical and subtropical areas. These areas have the similar temperature which is between 28°C and 30°C and it is suitable for mung bean to yield the optimal output (Karim, Fukamachi, & Hidaka, 2003). It serves as a high-consuming food source because approximate 90% of the mung bean production is sold to European nations, Australia and United States (Reddy et al., 2008).

Mung bean is categorized under Fabaceae family. It possesses diploid (2n) chromosomes. From the morphological view, mung bean is observed as taproot and nodules present. Its solid stem usually prostrate and hairy covered. Its branches are arching and the entire plant can grow up to 1 meter maximum. For leaves, they are all petiolate, compound, triangulate, hairy and green in color. Basically, the inflorescence of mung bean is short, axillary, racemes and with five to six flowers. Its elongated and straight fruits are hairy and consist of three to ten brown or black seeds (Encyclopedia of Life, 2016).

The life cycle of mung bean is around 60 days and this characteristic allows it to be harvested in a short period. Another contribution from mung bean is it plays the role as an effective helper in nitrogen fixation (Dewi & Fiatin, 2015). It can elevate the fertility of soils by assimilate the atmospheric nitrogen along with soil microbes. Hence, compared to other floras, its tolerance towards nutrient deficiency is higher (Dewi & Fiatin, 2015).

2.1.2 Usage of *V. radiata*

Mung bean is nutritionally rich in protein, carbohydrate and micronutrients (Dahiya et al., 2013). Based on the research finalized by Reddy et al (2008), the sprouts of mung bean contained ascorbic acid for human consumption. Ascorbic acid acts as a useful compound in preventing scurvy, degenerative diseases and mitigate gastric ulcers. Unlike animals, humans failed to biosynthesize ascorbic acid by themselves. Therefore, it is vital in order to maintain the normal growth of humans.

Therapeutically, mung bean is always used as a supplement as its amino acids are easy to be digested and causes less flatulence cases (Fery, 2002). In medical and cosmetic purposes, mung bean is useful owing to its antidotal action (Sharma et al., 2009). According to Kahraman, Adali, Mehmet, Onder and Nur (2014), it has antihypertensive and antidiabetic effects as well. Other medicinal benefits such as treatments in hepatitis, gastritis and heat rash characterize mung bean to be famous over the world (Kahraman et al, 2014). Antioxidants such as vitexin and isovitexin in mung bean soup are critically important to prevent injury from heat stress (Cao et al., 2011).

On the other aspect, mung bean is treated as a popular model for the research purposes. Its small genome size, self-pollination and fast growth rate catch the attention of scientists to further the application of it (Kim, Nair, Lee, & Lee, 2015). In order to explore the genetic enhancement and maximal production of mung bean, efforts such as molecular marker development and linkage maps are constructed in the breeding program (Kim et al., 2015). Developing nations can obtain benefits from this as malnutrition and starving issue can be solved with the optimal supply of mung bean.

2.2 OSMOTIC PRESSURE

2.2.1 Role of Abscisic Acid (ABA) and Its Function

ABA is categorized as one of the five essential natural plant growth regulators, including auxin, cytokinin, gibberellin and ethylene. It can be biosynthesized within the plant tissues and transported either within the route of phloem or via cell-to-cell transport (Wareing & Bradbeer, 1978).

It travels naturally in the plant body and provides several physiological responses such as leaf abscission, growth inhibition and degradative changes in senescence (Walton, Soofi, & Sondheimer, 1970). Besides, ABA is believed to take part in the nucleic acid metabolism. Particularly, Van Overbeek et al. (1967) demonstrated that ABA tended to inhibit the biosynthesis of nucleic acid, especially DNA, in Lemna. Another credit contributed by Villiers (1968) has showed the evidence that ABA inhibits the nucleotides uridine and thymidine incorporation into nucleic acids of Fraxinus embryos.

In addition, ABA inhibits the plant cell growth by its natural ability of modifying the cell membrane properties (Wareing & Bradbeer, 1978). It is comprehended as with the presence of ABA, potassium absorption may be mediated through inhibition of proton excretion by the cell Wareing & Bradbeer, 1978). Therefore, this causes the inhibition of segment development of the entire plant by the reduction of plant cell wall (Kutschera & Schopfer, 1986).

Under the high concentration of ABA, *Tradescantia virginiana* grew with smaller stomates, compared to the control group (Franks & Farquhar, 2001). The production of ABA will be slowly accumulated in the leaves subsequently resulting in the physiological changes including the