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Original Article

GERMINATION AND SEEDLING GROWTH OF SOME SELECTED AGRICULTURAL CROPS UNDER VARIOUS ABIOTIC STRESSES

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ABSTRACT

Objective: The aim of this investigation was to evaluate the germination response of *Vigna mungo*, *Vigna radiata*, Zea *mays* and *Brassica nigra* to different stress conditions.

Methods: The germination responses of four crops*, Brassica nigra, Vigna mungo, Vigna radiata* and *Zea mays* were studied under control conditions in the laboratory. The treatments included three light levels (red, green and white) and dark period for 24 to 48 h, three salinity concentrations (100, 300 and 500 mmol/l sodium chloride), temperature regimes of supra-optimal (35/28 °C), optimal (28/18 °C) and sub-optimal (15/08 °C) and heavy metals (cadmium, lead and nickel) treatments.

Results: Best seed germination of all these species was obtained in distilled water (control). The increase in concentration of salinity progressively inhibited the germination in all the selected plants. Light variedly affected seed germination. Seeds were subjected to white light shows the highest germination followed by red light. Darkness has no significant effect on seed germination in all the three plants. The seeds subjected to dark treatment showed better germination as well as a lower antioxidant activity than those subjected to various light conditions. Similarly, the seeds subjected to supra and sub-optimal temperatures shows inhibition were adversely affected with inhibited germination rates.

Conclusion: The present investigation reveals that among the four plants, *B. nigra* showed maximum tolerance against various stress conditions with maximum seed germination.

Keywords: Seed germination, Light, Temperature, Salinity

INTRODUCTION

Vigna mungo of family Fabaceae has been used for various medicinal purposes in ayurvedic and unani systems of medicine. The seeds are sweet, laxative, aphrodisiac, tonie, appetizer, diuretic, galactagogue and styptic useful in piles, asthma, scabies, leucoderma, gonorrhoea, pains, epistaxis, paralysis, rheumatism and affections of the nervous system, liver and cough [1]. *Vigna radiata* of family Fabaceae is well known for its detoxification activities and is used to refresh mentality, alleviate heat stroke and reduce swelling in the summer [2]. *Zea mays* is an annual crop that belongs to the family of grass i.e. Poaceae has been used to pacify kapha, pitta, anorexia, general deabilities, emaciation and haemorrhoids [3]. *Brassica nigra* of family Brassicaceae is used externally for many afflictions, like arthritis and rheumatism [4].

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Germination is regarded as phenomenons which commence with the uptake of water by a quiescent dry seed and terminates with elongation of the embryonic axis [5]. There are a number of various environmental factors that affects seed germination environmental factors regulating germination include temperature, water, and oxygen for non-dormant seeds along with light and the chemical environment for dormant seeds [6, 7]. Temperature, light, pH, salinity, water potential are the factors required for seed germination at an optimal proportion. Due to unconditional changes in the environment, all these parameters becomes stress condition for seed germination. Temperature has an impact on a number of processes that regulate seed germination including membrane permeability and the activity of membrane-bound and cytosolic enzymes [8]. The optimal germination temperature for most seeds which are not in dormancy is 25 to 30 °C. In addition water and temperature often interact in regulating seed germination. For example, seeds are capable of germinating at higher levels of water stress at optimum temperatures [9].

Light is also important for seed germination. Some species need a constant temperature and light to germinate, and others can germinate either under light or dark conditions but need temperature fluctuations. Salinity is one of the major abiotic stresses in most regions of the world. Salt damage to the seed germination is attributed to various factors such as reduction in water availability, changes in the mobilization of stored reserves and affecting structural organization of proteins [10-12]. In our study, we select the seeds of *Vigna mungo*, *Vigna radiata*, Zea *mays* and *Brassica nigra* due to their high nutrient, medicinal and commercial value. Therefore the objective of this study was to evaluate the germination response of above agricultural crops to different stress conditions.

MATERIALS AND METHODS

Seed source

The seeds of *B. nigra*, *Z. mays*, *Vigna radiata* and *Vigna Moong* were collected from Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India. It was dried and kept unsterilized in polyethene bags for future use.

Light sources

Different light treatments determined the effect of light on seed germination. White light provided by 40 W fluorescent tubes. Red and green were provided by 40W fluorescent tubes in combination with layers of red and green plastic sheet filters respectively. For dark treatment petri plates were covered with black sheets and kept in box, germination was monitored every 2 d under dim light.

Salinity treatments

Seeds were surface sterilized with 0.85% sodium hypochlorite (HIMEDIA) for 1 min. Six salinity concentrations (0, 100, 300and 500 mmol/l NaCl) were used based upon a preliminary test for salt tolerance of the species [13].

Physico-chemicals treatments

Temperature

Temperature influences plant growth and development through its effect on the rate of physiological and biochemical reactions. Thus, seeds of the selected plants were subjected to three different temperature: supra-optimal (35/28 °C), optimal (28/18 °C) and sub-optimal (15/08 °C).

Heavy metal treatments

Heavy metal treatments procedure followed by Aydinalp and Marinova 2009 [14]. Heavy metal test solutions were prepared from metal Cd, Ni and Pb in two different concentrations of 250 ppm and 500 ppm using chloride salts of respective metals. The chemical was of merck analytical reagent grade. Distilled water was used as a control. After soaking the selected seeds in respective treatment solutions for 24 h, seeds were subjected to standard germination test using three replications of 30 seeds each. Seeds were placed on two sheets of Whatman No.1 filter paper contained in trays (30x20 cm size). Solutions of heavy metal (30 ml) and distilled water as control were subsequently added to the corresponding treated seeds to wet the filter paper and to maintain the contaminated environment. Each treatment was replicated three times. The trays containing treated seeds were incubated at room temperature of 28±2 °C and the experiment lasted for 12 d. Germination was considered to have occurred when radicles were 2 mm long. Germination percent was recorded every 24 h till the end of the experiment.

Antioxidant assay

Catalase activity was analyzed following the method proposed by Luck *et al.* (1974) [15] 20% homogenate was preparedin phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. Phosphate buffer (3.0 ml) was taken in an experimental cuvette followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

Assay of peroxidase (POD): 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the various parts of the plant, clarified by centrifugation and the supernatant was used for the assay. To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added, and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 min in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm [16].

Statistical analysis

All experiments were carried out in triplicate. Data are presented as arithmetic means and standard deviation. Differences in the majority of plant biometric variables were sought using one-way ANOVA. All calculations were performed using SPSS version 13.00 software (Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

Seed germination of crops (*B. nigra, V. mungo, V. radiata* and *Z. mays)* is regulated by factors such as water, temperature, light, soil salinity and their interactions. However, each species responds to the abiotic environment in a unique manner. Agricultural crops vary notably in their ability to tolerate salt and this variation may due to variation in temperature regimes and availability of light.

Effect of salinity on seed germination

There was a reduction in seed germination percentage with an increase of salinity concentrations in the growing medium. Germination was greatly reduced in the 500 mmol/l NaCl (fig. 1). Highest germination percentage of all the seeds was observed in distilled water (control) at room temperature (20 °C-30 °C). Similar results were observed by a number of previous authors like [17-22] under various treatments of salt stress.

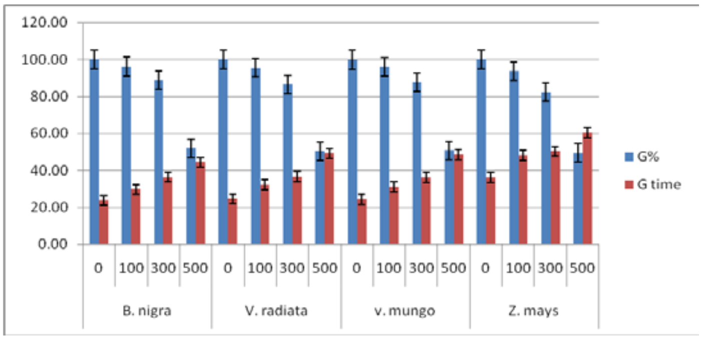

Fig. 1: Effect of salinity on germination time and percentage of the selected plants
Data are given in mean±SEM, n=3

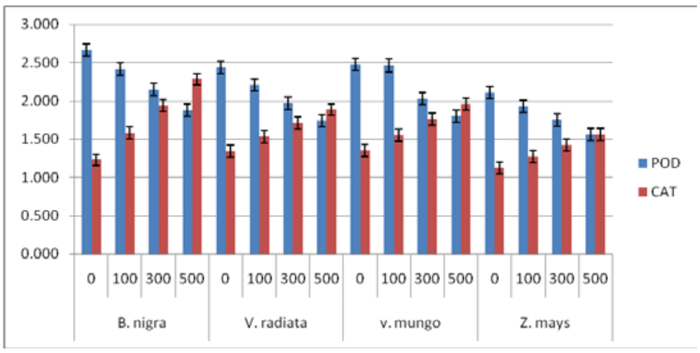

Fig. 2: Effect of salinity on POD and CAT activity of the selected plants
Data are given in mean±SEM, n=3

Effect of light on seed germination

The present study revealed that the maximum germination percentage was observed in white light (90) followed by red light (85%) in and minimum germination observed in green light (50%). The highest germination was seen in seeds kept in dark conditions 24–48 h (fig. 3).

Effect of temperature

Seeds treated with sub and supra-optimal temperatures took longer germination time than that of seeds at room temperature (20 °-30 °C). In the case of *Zea mays* germination was reduced as compared to *V. mungo, V. radiata* and *B. Nigra* (fig. 5.). When the temperature exceeds the optimum for biological processes crops often respond negatively with a reduction in net growth. This was observed in maize where an increase in temperature from the optimum (25-30°C) to 35°C resulted in a reduction in seedling growth [23, 24]. Wolfe (1991) [25] found that the relative growth rate of maize was decreased by 10% when the temperature was reduced from 28/18°C to 18/13°C. In other cases, an increase in temperature was reported to accelerate seed germination and its growth [26, 27].

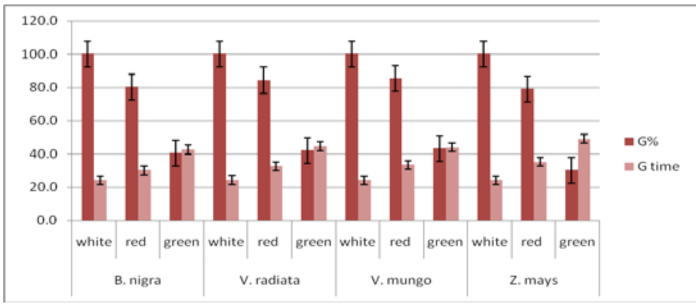

Fig. 3: Effect of light on germination time and percentage of selected plants
Data are given in mean±SEM, n=3

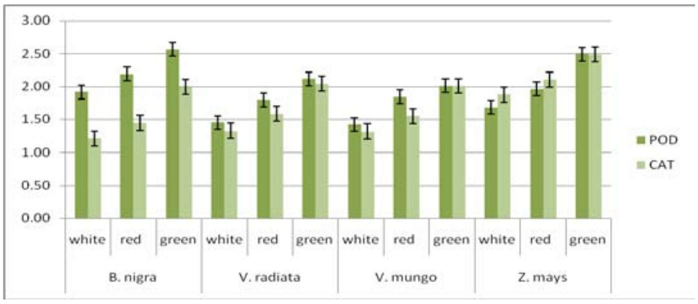

Fig. 4: Effect of light on POD and CAT activity
Data are given in mean±SEM, n=3

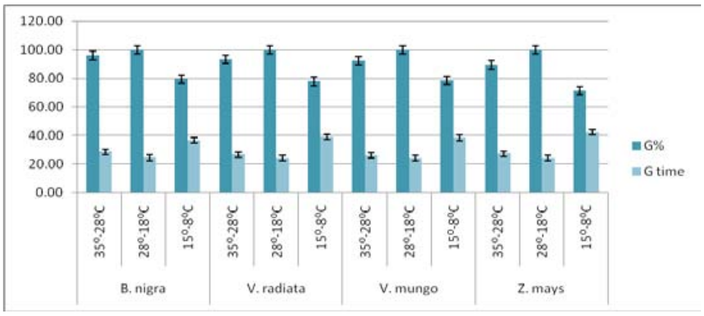

Fig. 5: Effect of temperature on germination time and percentage of selected plants
Data are given in mean±SEM, n=3

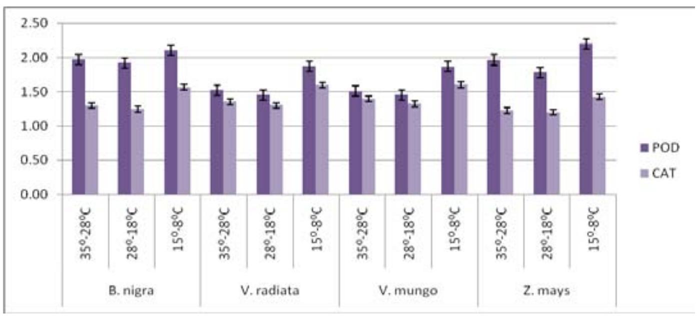

Fig. 6: Effect of temperature on POD and CAT activity
Data are given in mean±SEM, n=3

Antioxidant assay and protein content

The results of the present study showed all the tested concentrations of NaCl caused oxidative stress in *B. nigra, V. mungo, V. radiata* and *Z. mays*. As NaCl concentrations increased CAT and POD activity enhanced progressively. The decline in antioxidant enzyme activity at a higher salt concentration(s) and low temperature may result from the inactivation of the enzyme by H_2O_2, which is produced in different cellular compartments or from a number of non-enzymatic and enzymatic processes in cells [28].

There was no significant effect of light on oxidative enzymes activity and protein content. There are data that peroxidase catalyzes the oxidative coupling of phenols thus which binding molecules of polysaccharides and glycoproteins [29]. This results in changes of the physical properties of cell walls. Peroxidases are able to reduce cell wall extension rate by catalyzing the formation of covalent bonds such as isodityrosine or diferulic acid bridges [30] and by oxidizing cinnamic alcohols to radicals which polymerize to lignin [31] and this could be the reason behind inhibited germination in the seeds of selected plants.

Heavy metals

The effect of various heavy metals (Cd, Pb and Ni) on the germination percent of selected plants is shown in the fig. 7. The percent seed germination was highest in the seeds grown in distilled water i.e. control. However, Cd, Pb and Ni treatments reduced seed germination in all the four plants at all the selected concentrations (100, 300 ppm and 500). The inhibitory effects of heavy metals were more prominent at higher doses. The germination percent was remarkably reduced in 500 ppm of Cd, Pb and Ni.

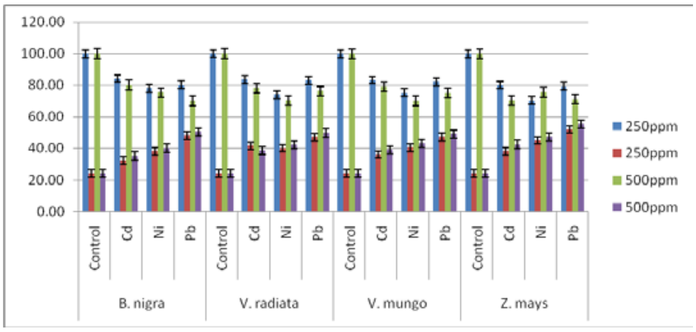

Fig. 7: Effect of heavy metals on germination time and percentage of selected plants
Data are given in mean±SEM, n=3

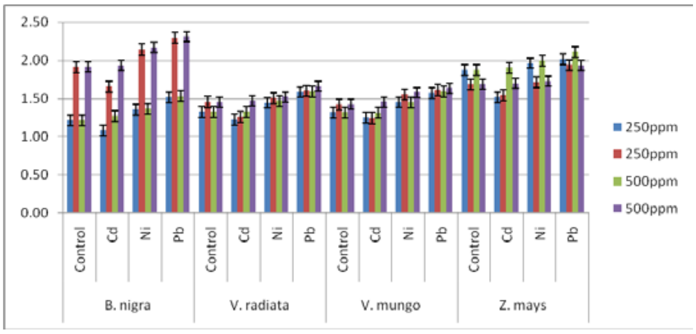

Fig. 8: Effect of heavy metals on POD and CAT activity
Data are given in mean±SEM, n=3

The present study indicates that the seeds of the selected plants observed stress at temperatures lower or higher than the optimum one. The test species differed greatly in their response to salinity as quantified by germination percentage. Best germination was obtained in controls in all the species. The increase in salinity progressively inhibited germination, few seeds of in Z. *mays* germinated above 300 mmol/l NaCl. The seeds of other species showed some germination even at 500 mmol/l NaCl.

The increase in salinity resulted in decreased seed germination in all the plant species. The increase in the concentration of stress enzymes further supports the idea of inhibited seed germination and subsequent stress in the seeds at extreme ends of various abiotic factors. Beside that the metals at higher doses also inhibited germination. Among the four plants *B. nigra* showed best tolerance towards heavy metals stress. However, it was quite sensitive to the supra and sub-optimal temperatures. Compared to salinity, light treatments (white, red and green) caused mild stress in the seeds subjected to various treatments which we previously found caused a slight decreased reduction in germination percentage of these selected species. Seed germination traits of these selected species showed that the highest dose of salt, varied low temperatures reduced seed germination as well as delayed germination time compared to control. The results are compatible with findings of many researchers [32, 34].

Ultimately, reduction in seed germination can be described by higher salinity proportions and other temperature and light stresses [35]. Elevated salinity reduced water uptake by plant thereby inhibits root elongation [36]. Environmental factors such as high temperature, drought, salinity and heavy metal have decisive effects on seed germination, growth, development, and production.

Furthermore the results of the present study clearly revealed that heavy metals unfavorably influenced the seed germination in the selected plants. Observations of this study is in conformity with the previous findings of Seyed *et al.*[37] who reported that germination rate was decreased in Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and Safflower (*Carthamus tinctorious*) with an increase in the concentration of Cd^{2+}. Reduction in seed germination may be due to the interference and alterations in the cell membrane permeability properties by Cd^{2+} which resulted in decreased water absorption and transport as well as lowered water stress. Similar reduction in seed germination was observed by Sharma et. al. [38] in *B. juncea* under Zn, Mn, Ni and Co treatments.

CONCLUSION

Thus, the present study clearly indicates the growing threats to agricultural productions resulting from the drastically changing global climate as a result of blooming industrialization. Among the various side effects of climate change increase in temperature is one of the major issues. Besides that one of the major problems affecting the seed germination and productivity of plants is heavy metal stress. The investigation result clearly reveals the inhibitory effects of heavy metals with respect to seed germination in all the selected plants. Furthermore, the intensity of inhibition was directly proportional to the concentration doses of heavy metals employed. High doses of salinity and heavy metals were found responsible for decreasing the percentage of seed germination in all the four plants. Result of the present study also indicates that deviations from the standard treatments caused an enhancement in the production and activity of antioxidant enzymes compared to healthy control seedlings which clearly indicates that seedling growth has been unfavorably affected by the applied stress conditions. Among the four plants, *B. nigra* showed maximum tolerance against various stress conditions with maximum seed germination.

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CONFLICT OF INTERESTS

Declare none

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