

## Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*)

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**Abstract:** An experiment was conducted to see the impact of osmotic stress as it is one of the main cause in various soil and water disorders in agricultural field crops, specially the seed germination and seedling growth. The osmotic stress was generated using PEG-6000 and the seed germination, seedling growth were evaluated including the status of pigments i.e. chlorophyll (a, b and total), total carotenoids, pheophytin (a, b and total) and different enzymes like amylase, peroxidase, catalase and superoxide dismutase. The various osmotic potentials generated (-2, -5 and -10 bars) showed significant decrease in germination percentage as at the osmotic potential of -10 bars it was observed 70 in comparison to 90% of control. All the seedling growth parameter also showed inhibition with increase in osmotic potential. Increase in osmotic stress decreased Chlorophyll 'a', while Chlorophyll 'b' was increased in -5 bars while total chlorophyll showed decrease in -5 bars osmotic potential. Total carotenoids and pheophytin (a, b and total) were highly increased in -5 bars and decreased in -10 bars osmotic concentration. Enzymatic activity was found to be decreased in amylase while peroxidase, catalase and SOD were increased at different osmotic gradients in comparison to control. The data observed in the experiment can be helpful to assess the impact of any kind of osmotic stress on plant growth and development in crops.

**Key words:** PEG-6000, Osmotic potential, Seed germination, Seedling growth, *Phaseolus mungo*  
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### Introduction

Seed germination and early seedling growth are considered the most critical phases for establishment of any species. Germination of seed is strongly influenced by variation in temperature, water stress and light requirement and these factors often show significant interaction in their effects on germination (Hampson and Simpson, 1990). Water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. It is believed that these stress induced proteins allow plants to make biochemical and structural adjustments that enable plants to cope with the stress.

All the plants have an inbuilt ability to adjust to environmental variables. Abiotic stress negatively influences survival grain yield biomass accumulation and production of most crops (Khush and Baenziger, 1998; Grover *et al.*, 2001; Bhattacharjee, 2008; Ozdener and Kutbay, 2008). Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] is an important and legume tolerant to drought and high temperature (Kharb *et al.*, 1987; Kumar and Singh, 2002). Large number of drought induced genes has been identified in a wide range of plant species (Bray, 2002). In sandy soils, where moisture retention is poor, the development of stress is rapid and soil moisture deficit adversely influences the metabolism, growth and yield of crops.

Vyas *et al.* (1996) reported that increasing water stress progressively decreased the activities of nitrate reductase, glutamine synthetase and glutamate synthase, and soluble protein content in

moth bean leaves. Garg *et al.* (2001) also found that increasing water stress progressively decreased plant water potential, leaf area, net photosynthetic rate, starch and soluble protein contents and nitrate reductase activity in moth bean genotypes. In several arid zone crops genotypic differences in response to water stress exist (Garg *et al.*, 1998; Kuhad and Sheoran, 1986). The reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Chaves *et al.*, 2003).

Global climate change, in the form of rising temperature and altered soil moisture, is projected to decrease the yield of food crops over the next 50 years (Thomson *et al.*, 2005). Global changes include mainly temperature rise and water deficiency. The lack of moisture in soil directly affects seed germination and seedling growth in plant. The present experiment was planned to correlate the moisture status in reference to seed germination and seedling growth in black gram (urd) through generating osmotic stress using PEG-6000 in petridish culture experiment.

### Materials and Methods

Research work was carried out in petridishes in the laboratory of Plant Physiology of Lucknow University during the year 2008. The seeds of black gram (*Phaseolus mungo* c.v. type-9) were screened for osmotic stress. To provide variable osmotic stress the different concentrations of polyethylene glycol-6000 (PEG) were prepared to obtain solutions of different osmotic potentials

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following the methods of Michel and Kaufmann (1973). The water stress levels (solutions) used as germination medium was -2 bars, -5 bars and -10 bars.

Seeds of black gram (urd) were sterilized by immersing in 1%  $\text{HgCl}_2$  for 5 min and rinsing repeatedly with distilled water. They were then germinated in petridishes (65 mm) containing a sheet of filter paper (Whatman No. 1), moistened with 1 ml of distilled water (control) or polyethylene glycol (-2, -5 and -10 bars PEG test solutions). After 14 hr, 5 ml of each concentration of nutrient solution was added in respective petridish in diffused light and temperature ranging between 25-30°C.

The growth parameters like germination percentage, length of stem and root, number of lateral roots, fresh and dry weight were undertaken. The moisture percentage in seedlings was also calculated through fresh and dry weight of whole seedlings. For the dry weight, seedlings were placed in oven at  $70 \pm 5^\circ\text{C}$  till weight is constant.

For the estimation of pigments (1 gm) fresh leaves were extracted with (10 ml) 80% acetone and centrifuged at  $5000 \times g$  for 10 min and filtered by Whatman filter paper no. 1. Chlorophyll (a, b and total), carotenoids and pheophytin (a, b and total) were estimated as per method of Arnon (1949) as amended by Lichtenthaler (1987). Finally the extraction and determination were performed spectrophotometrically (Double Beam UV-VIS Spectrophotometer UV 5704 SS) and the result were expressed as  $\text{mg g}^{-1}$  EC fresh wt.

For the amylase estimation, extract of seedling (2.5%) was prepared in 10 ml distilled water in mortar and pestle using a pinch of acid wash sand under darkness and low temperature, and used for the estimation of amylase activity ( $\alpha$ ,  $\beta$  and total) in terms of mg starch hydrolyzed per gm fresh weight of tissue by the method of Katsuni and Fukuhara (1969). For each sample extract was divided into two parts, one part was heated at  $70^\circ\text{C}$  for 15 minute to destroy  $\beta$ -amylase while second part kept unheated. For sample the reaction mixture was prepared using 2 ml buffer (Citrate phosphate buffer pH -5.5), 1 ml distilled water, 1 ml enzyme extract and 1 ml 0.5% starch solution. After 30 minute of incubation at room temperature 1 ml 1N  $\text{H}_2\text{SO}_4$  was added to stop the reaction. For blanks in the reaction mixture in 1 ml 1N  $\text{H}_2\text{SO}_4$  was added before starch solution, while remaining process was same. From this reaction mixture took 2 ml supernatant from each test tube separately. In each test tube 1 ml  $\text{I}_2 + \text{KI}$  solution was added and final volume was made 25 ml by adding distilled water. The optical density was measured at the wavelength 620 nm with the help of spectrophotometer.

The catalase activity was measured by the method of Euler and Josephson (1927) in 10 ml reaction mixture, standardized against 0.1N  $\text{KMnO}_4$  containing 0.5 mM  $\text{H}_2\text{O}_2$  and 1 mM phosphate buffer, pH 7.0, stabilized at  $25^\circ\text{C}$ . The reaction was initiated by adding 1 ml of suitably diluted enzyme extract to the reaction mixture and was allowed to proceed for 5 min after which 5 ml of 2N  $\text{H}_2\text{SO}_4$  was added to stop the reaction. Corresponding blanks were run

simultaneously in which sulfuric acid was added prior to the addition of enzyme extract. The amount of  $\text{H}_2\text{O}_2$  reduced by the enzyme was determined by titrating the reaction mixture against 0.1 N  $\text{KMnO}_4$ . After making the blank corrections, results have been expressed as  $\mu\text{M H}_2\text{O}_2$  decomposed.

The peroxidase was assayed by the method of Luck (1963). The assay system contained 5 ml 0.1 M phosphate buffer (pH 6), 1 ml 0.01% (v/v)  $\text{H}_2\text{O}_2$  and 1.0 ml 0.5% p-phenylenediamine. The reaction was run by the addition of suitably diluted enzyme extract at  $25^\circ\text{C}$  for 5 min and was stopped with 2 ml 5N  $\text{H}_2\text{SO}_4$ . A blank with added  $\text{H}_2\text{SO}_4$  was also taken. After centrifugation, OD of the supernatant was measured at 485 nm. The change in OD of  $0.01 \text{ min}^{-1}$  represents one unit of peroxidase activity.

Superoxide dismutase activity was measured by the photochemical method as described by Giannopoliti and Ries (1977) with slight modification. The reaction mixture consisted of 20 mM sodium phosphate buffer pH 7.5, 0.1 mM EDTA and 10 mM methionine, 0.1 mM p-nitroblue tetrazolium chloride (NBT) in ethanol, 0.005 mM riboflavin and enzyme extract. Blanks were kept in the dark and others were illuminated for 30 min. Total SOD activity was defined as the amount enzyme required to causes 50% inhibition of the rate of NBT reduction at 560 nm.

**Statistical analysis:** Each treatment was analyzed with at least three replicates and standard deviation (SD) was calculated. Statistical analysis was performed using the Student's *t*-test;  $p < 0.05$  and  $p < 0.01$  was considered statistically significant.

## Results and Discussion

The overall findings in the present experiment clearly show the effect of osmotic stress on seed germination, seedling growth, pigments and enzymatic activities in petridish culture.

Germination of black gram seeds as shown in Table 1 was 90% in control and there was highest decline in germination percentage with decrease in osmotic potential in -2 bars with (85%), -5 bars (75%), -10 bars (70%) in respect to control in black gram. The length of stem, root and number of lateral roots were decreased with increase in PEG (polyethylene glycol) concentration. The fresh weight also decreased with increase in PEG concentration. Dry weight was found to be decreased in -10 bars while increased in -2 bars PEG concentration. The moisture percentage was found highly increased in -5 bars PEG while it decreased in -10 bars PEG concentration.

Osmotic stress caused a considerable decrease in germination. Similar declines in seed germination have been reported in the literature (Khan and Ungar, 1997; Woodell, 1985; Gupta *et al.*, 1993; Singh *et al.*, 1996; Ungar, 1996). Reduced germination under water stress conditions may be attributed to the effect that seeds seemingly develop an osmotically enforced "dormancy" under water stress conditions, which may be an adaptive

**Table - 1:** Effect of osmotic stress on seed germination and seedling growth in 15 d black gram seedlings

Treatments	Germination (%)	Length of stem (cm)	Length of root (cm)	No. of lateral roots plant <sup>-1</sup> (cm)	Fresh wt. plant <sup>-1</sup> (gm)	Dry wt. plant <sup>-1</sup> (gm)	Moisture (%)
Control	90	7.7773±0.4746	7.366±0.5686	11.111 ± 0.4844	0.2616±0.0080	0.0496±0.0020	81.039
PEG (-2 bars)	85	6.4443±0.8183*	6.866±0.6544	8.444 ± 0.6185	0.2353±0.0176	0.0499±0.0038	78.793
PEG(-5 bars)	75	5.8886±0.2420	6.627±0.5138	8.444 ± 0.8678*	0.2296±0.0253	0.0469±0.0005	79.573
PEG (-10 bars)	70	3.8886±0.2001	5.811±0.1159	3.888 ± 0.6938*	0.2216±0.0062	0.0473±0.0029	78.655

\* = Significant at 0.05 level, Values are mean of triplicate ± SD

**Table - 2:** Effect of different osmotic levels on leaf pigments (in mg g<sup>-1</sup> fresh wt. of tissue) in 15 d old black gram

Treatments	Chl 'a'	Chl 'b'	Tot. chlorophyll	Total contenoids	Pheophytin 'a'	Pheophytin 'b'	Total pheophytin
Control	0.885±0.001	0.3696±0.001	1.316±0.001	0.3700±0.002	1.239±0.001	0.7946±0.003	2.031±0.0040
PEG (-2 bars)	0.945±0.001	0.3746±0.002	1.381±0.005	0.3790±0.002	1.426±0.002	0.9806±0.009	2.404±0.0078
PEG(-5 bars)	0.017±0.003	0.9730±0.004	0.895±0.000	0.3956±0.000	1.392±0.000	1.1523±0.005	2.941±0.0049
PEG (-10 bars)	0.011±0.000	0.0633±0.000	0.923±0.000	0.2576±0.001	0.634±0.005	0.9493±0.001	1.552±0.0055

\* = Significant at 0.05 level, Values are mean of triplicate ± SD

**Table - 3:** Effect of different osmotic levels on enzymes in 15 d old black gram seedlings

Treatments	Amylase	Peroxidase	Catalase	SOD
Control	2.213 ± 0.035	2.653 ± 1.092*	9.333 ± 1.333*	0.791 ± 0.0017
PEG (-2 bars)	2.133 ± 0.148	3.293 ± 1.139*	29.333 ± 8.743**	0.839 ± 0.0045
PEG(-5 bars)	1.226 ± 0.026	3.306 ± 0.724	154.666 ± 12.719**	0.847 ± 0.0020
PEG (-10 bars)	0.813 ± 0.013	4.506 ± 2.075**	224.0 ± 18.037**	0.857 ± 0.0232

\* = Significant at 0.05 and \*\*0.01 levels, Values are mean of triplicate ± SD, SOD = Super oxide dismutase, Amylase activity in mg starch hydrolyzed g<sup>-1</sup> fresh tissue weight, Peroxidase in ΔO.D. g<sup>-1</sup> fresh tissue weight, Catalase in ml H<sub>2</sub>O<sub>2</sub> hydrolysed g<sup>-1</sup> fresh weight, SOD units mg<sup>-1</sup> dry weight

strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Singh *et al.*, 1996; Tilki and Dirik, 2007).

Osmotic stress caused a significant reduction in water uptake resulting into low water contents in germinated embryos and endosperm was observed, indicating that these tissues were under stress. Similar observations of decreases in water level under stress conditions were made by Gill *et al.* (2001) in *Sorghum spp.*, Siddique *et al.* (2000) in wheat, Prado *et al.* (2000) in *Chenopodium spp.* The germinability of seeds decreased with increasing the level of water stress. Not only the germination was inhibited, but the extension growth of the seedlings was also obstructed. These results show that radicle and plumule growth of the seedlings was greatly adversely affected by water stress.

Slow and poor germination under water stress is obviously due to decreased water potential of the germination medium, which restricts the water availability to the seeds (Uniyal and Nautiyal, 1998; Soltani *et al.*, 2002). Reduction in germination at higher level of moisture stress may be attributed to the moisture deficit in the seeds below the threshold, which may lead to degradation and inactivation of the essential hydrolytic and other group of enzymes as suggested by Wilson (1971). As water moisture is one of the primary requirement in seed germination (Evenari, 1980, 1981) the water stress developed by PEG reduced germination greatly in black gram. Similar results were obtained by Singh and Singh (1981 a, b) in

different plants in both laboratory as well as field conditions in relation to increase in moisture stress. Previously Hadas (1976) and Hadas and Stibbe (1973) in *Viscia faba* have also shown the importance of water as a limiting potential (lower than - 30 bars) as repressive for seed germination. In earlier research, Gill and Singh (1985) have reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stress.

In relation to growth during germination both embryo and endosperm growth was suppressed by osmotic treatment. They were smaller than in distilled water because of reduced fresh weight, resulting from reduced water absorption (Prado *et al.*, 1995). The increase of embryos fresh weight in distilled water was mainly due to an increase of tissue water content which was reflected from (fresh wt. - Dry wt. / Fresh wt.) x100 values (Table 1). This increase was not significant in endosperm where the (fresh wt./dry wt.) ratio showed no significant variation. Osmotic adjustment has been shown to reduce growth sensitivity to water stress (Cutler *et al.*, 1980) or to allow growth to proceed at a lower rate under water stress (Meyer and Boyer, 1981) by maintaining turgor. Thus, it can be concluded that growth at lower water potential was a result of turgor maintenance, whereas the inhibition of growth was not entirely dependent on turgor (Bassiri Rad and Coldwell, 1992). In contrast to a significant decrease in FW, stress imposition resulted in a significantly higher gain in biomass in germinating embryos and endosperm as is shown from an increase in DW values after osmotic stress treatment. This osmotic stress induced

DW increase, principally in germinated embryos, might be attributed to the increased synthetic activity associated with cell division and new material synthesis (Sunderland, 1960).

The results regarding the effect of osmotic stress on pigment chlorophyll (a, b and total), total carotenoids, pheophytin (a, b and total) in 15 days old black gram (a C3 plant) seedlings are shown in Table 2. Effect of osmotic stress on pigment status in chlorophyll 'a' showed decrease with increase in PEG concentration. Chlorophyll 'b' increased maximum in -5 bars and lowest decrease in -10 bars, total chlorophyll increased in -2 bars and decreased in -5 bars PEG concentration. Total carotenoids, pheophytin (a, b and total) were highly increased in -5 bars, and decreased in -10 bars PEG concentration. The Effect of osmotic stress on enzymes amylase, peroxidase, catalase and SOD in black gram seedling is shown in Table 3. Amylase was found to be decreased with increase in PEG concentration. Peroxidase, catalase and SOD increased at different gradients in comparison to control.

Chlorophyll contents decreased significantly as the stress intensity increased with greater impact on black gram. In rice chlorophyll concentration was decreased (Agarwal and Mehrotra, 1978) but showed increased concentration of carotenes and pheophytin. Decreased in plant water status was associated with significant decline in net photosynthetic rates in Urd. Decreased plant water potential and RWC under drought leading to reduce leaf turgor (Sen Gupta *et al.*, 1989) might be responsible for the reduction in photosynthesis observed in the present study. Garg *et al.* (2001) have also observed significant differences in rate of net photosynthesis under increasing water stress in some moth bean genotypes.

The decrease in antioxidants with increase in stress level was notable in black gram. Previously, a decrease in the level of antioxidants was observed with increase in stress intensity in wheat by Zhang and Kirkham (1994). Amylase and its important role during seed germination through hydrolysis of reserve starch and release in the energy has been worked out by Nauriere *et al.* (1992). The amylase activity in seedlings under the influence of high levels of PEG was found to be decreased in comparison to control. Enzymes activities showed great variation with increasing level of osmotic stress. Where as activity of total,  $\alpha$  and  $\beta$  amylase was decreased, activities of catalase, peroxidase and SOD was increased by these chemicals. Decreased activity of amylase could be explained decreased moisture and reserved materials in the treated plants.

Catalase is another important antioxidant enzyme that converts  $H_2O_2$  to water in the peroxisomes (Fridovich, 1989; Mc Cord and Friovich, 1969). In this organelle,  $H_2O_2$  is produced from  $\beta$ -oxidation of fatty acids and photorespiration (Morita *et al.*, 1994). Higher activities of catalase decreased  $H_2O_2$  level in cell and increase the stability of membranes and  $CO_2$  fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to  $H_2O_2$ . A high level of  $H_2O_2$  directly inhibits  $CO_2$  fixation

(Yamazaki *et al.*, 2003). Increased catalase and peroxidase activities are indicative of redox induce oxidative stress induced by these chemicals.  $H_2O_2$  is products of  $O_2$  reduction which can explain reduce photosynthesis, hence less growth. Disturbed chlorophyll concentration, induces excess light induced over reduction of oxygen thus causing  $H_2O_2$  production, hence, as a defence mechanism catalase and peroxidase might scavenging  $H_2O_2$  to protect the plant. Such reports were known from various laboratories (Zelitch and Ochoa, 1953; Willkenes *et al.*, 1994).

Superoxide dismutase (SOD) catalyses the conversions of superoxide anions to hydrogen peroxide and water. Several reports have shown that over-expression of superoxide dismutase leads to increased tolerance to abiotic stress such as low temperature and water stress (reviewed in Bohnert and Sheveleva, 1998). Esfandari *et al.* (2007) Candan and Tarhan (2003), Martinez *et al.* (2001), Scandalios (1993), Sen Gupta *et al.* (1993) and Zhao *et al.* (2006) had similar findings and expressed that the increase the SOD activity and decreased in oxidative damage were closely related. In conclusive results it may therefore be suggested that black gram are sensitive to all these kinds of treatments, but the degree of impact varies in this plant.

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#### References

- Agarwal, S.C. and N.K. Mehrotra: Growth and metabolisms of rice plants subjected to high alkalinity (SAR) in irrigation water and soil calcareousness. *Ind. J. Plant Physiol.*, **21**, 59-65 (1978).
- Arnon, D.I.: Copper enzyme in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1-5 (1949).
- Bassiri Rad, H. and M.M. Coldwell: Root growth, osmotic adjustment and  $NO_3^-$  uptake during and after a period of drought in *Artemisia tridentata*. *A. Aust. J.K. Plant Physiol.*, **19**, 493-500 (1992).
- Bhattacharjee, Soumen: Triadimefon pretreatment protects newly assembled membrane system and causes up-regulation of stress proteins in salinity stressed *Amaranthus lividus* L. during early germination. *J. Environ. Biol.*, **29**, 805-810 (2008).
- Bohnert, H.J. and E. Sheveleva: Plant stress adaptations, making metabolism move. *Curr. Opin Plant Biol.*, **1**, 267-274 (1998).
- Bray, E.A.: Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: An analysis using microarray and differential expression data. *Ann. Bot.*, **89**, 803-811 (2002).
- Candan, N. and L. Tarhan: The correlation between antioxidant enzyme activities and lipid peroxidation levels in *Mentha pulegium* organs grown in  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Mn^{2+}$  stress conditions. *Plant Sci.*, **163**, 769-779 (2003).
- Chaves, M.M., J.P. Maroco and J. Pereira: Understanding plant responses to drought from genes to the whole plant. *Funct. Plant Biol.*, **30**, 239-264 (2003).
- Cutler, J.M., K.M. Shahan and P.L. Steponkus: Influences of water potentials and osmotic adjustment on leaf elongation in rice. *Crop Sci.*, **20**, 314-318 (1980).

- Esfandiari, E., M.R., Shakiba, S. Mahboob, H. Alyari and M. Toorichi: Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seeding. *J. Food Agric. Environ.*, **5**, 149-153 (2007).
- Euler, H., W. Voa and K. Josephson: Uberkatalase I. Leibigs Ann., 452, 158-181 (1927).
- Evenari, M.: The history of germination research and the lesson it contains for today. *Isreal J. Bot.*, **29**, 4-21 (1980-1981).
- Fridovich, I.: Superoxide dismutases: An adaptation to a paramagnetic gas. *J. Biol. Chem.*, **264**, 7761-7764 (1989).
- Garg, B.K., S. Kathju and U. Burman: Influence of water stress on water relations, photosynthetic parameters and nitrogen metabolism of moth bean genotypes. *Biol. Plant*, **44**, 289-292 (2001).
- Garg, B.K., S.P. Vyas, S. Kathju and A.N. Lahiri: Influence of water deficit stress at various growth stage on some enzymes of nitrogen metabolism and yield in clusterbean genotypes. *Ind. J. Plant Physiol.*, **3**, 214-218 (1998).
- Giannopolities, C.N. and S.K. Ries: Superoxide dismutasees. *Plant Physiol.*, **59**, 309-314 (1977).
- Gill, K.S. and O.S. Singh: Effect of salinity carbohydrate metabolism during paddy (*Oryza sativa*) seed germination under salt stress condition. *J. Exp. Biol.*, **23**, 384-386 (1985).
- Gill, P.K., A.D. Sharma, P. Singh and S.S. Bhullar: Effect of various abiotic stress on the growth, soluble sugars and water relations or sorghum seedlings grown in light and darkness. *Bulg. J. Plant Physiol.*, **27**, 72-84 (2001).
- Grover, A., A. Kappor, S.O. Lakshmi, S. Agarwal, S.K. Agarwal, M. Agarwal and H. Dubey: Understanding molecular alphabets of the plant abiotic stress responses. *Curr. Sci.*, **80**, 206-216 (2001).
- Gupta, A.K., J. Singh, N. Kaur and R. Singh: Effect of polyethylene glycol induced water stress on germination and reserve carbohydrates metabolism in Chickpea cultivars differing in tolerance to water deficit. *Plant Physiol. Biochem.*, **31**, 369-378 (1993).
- Hadas, A. and E. Stibbe: Physical aspects of soil water and salts in ecosystem. *Ecol. Stud.*, **4**, 97-106 (1973).
- Hadas, A.: Water uptake and germination of leguminous seeds under changing external water potential in osmoticum solutions. *J. Exp. Bot.*, **27**, 480-489 (1976).
- Hampson, C.R. and G.M. Simpson: Effect of temperature, salt and osmotic potential in early growth of wheat (*Triticum aestivum*) germination. *Can. J. Bot.*, **68**, 524-528 (1990).
- Katsuni, M. and M. Fukuhara: The activity of amylase in shoot and its relation to Gb induced elongation. *Physiol. Plant*, **22**, 68-75 (1969).
- Khan and Ungar: Effect of thermoperiod on recovery of seed germination of halophytes from saline condition. *Ame. J. Bot.*, **84**, 279-283 (1997).
- Kharb, R.P.S., V.P. Singh and Y.S. Tomer: Moth bean [*Vigna aconitifolia* (Jacq.) Marechal]- A review. *Forage Res.*, **13**, 113-132 (1987).
- Khush, G.S. and P.S. Baenziger: Crop improvement emerging trends in rice and wheat. In: Crop productivity and sustainability shaping the future (Eds.: V.L. Chopra, R.B. Singh and A. Verma). Oxford and IBH Publishing, New Delhi. pp. 113-125 (1998).
- Kuhad, M.S. and I.S. Sheoran: Physiological and biochemical changes in clusterbean (*Cyamopsis tetragonoloba* L.) genotypes under water stress. *Ind. J. Plant Physiol.*, **29**, 46-52 (1986).
- Kumar, D. and N.B. Singh: Moth bean in India. Scientific Publishers, Jodhpur (2002).
- Lichtenthaler, H.K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In: Plant cell membrane (Eds.: L. Packer and R. Douce). Vol. 148, Academic Press, New York. pp. 350-382 (1987).
- Luck, H.: Peroxidase. In: Methods of enzymic analysis (Ed.: H.U. Bergmeyer). Academic Press, New York. pp. 895-897 (1963).
- Mc Cord, J.M. and I. Fridovich: Superoxide dismutase: An enzymic function for reythrocuprein (Hemocuprein). *J. Biol. Chem.*, **244**, 6049-6055 (1969).
- Martinez, C.A., M.E. Loureiro, M.A. Oliva and M. Maestri: Differential responses of superoxide dismutase in freezing resistant *Solanum tuberosum* subjected to oxidative and water stress. *Plant Sci.*, **160**, 505-515 (2001).
- Meyer, R.F. and J.S. Boyer: Osmoregulation, solute distribution and growth in soybean seedlings having low water potentials. *Planta*, **151**, 482-489 (1981).
- Michel, B.E. and M.R. Kaufmann: The osmotic potential of polyethylene glycol 6000. *Plant Physiol.*, **51**, 914-916 (1973).
- Morita, S., M. Tasake, H. Fujisawa, T. Ushimaru and H. Tsuji: A cDNA clone encoding a rice catalase isozyme. *Plant Physiol.*, **105**, 1015-1016 (1994).
- Nauriere, C., C. Doyen, C. Thevenot and J. Dauffant:  $\beta$ -amylase in cereals: Study of the maize  $\beta$ -amylase system. *Plant Physiol.*, **100**, 887-893 (1992).
- Ozdener, Yasemin and H. Guray Kutbay: Effect of salinity and temperature on the germination of *Spergularia marina* seeds and ameliorating effect of ascorbic and salicylic acids. *J. Environ. Biol.*, **29**, 959-964 (2008).
- Prado, F.E., C. Boero, M. Gallardo and J.A. Gonzalez: Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* wild seeds. *Bot. Bull. Acad. Sin.*, **41**, 27-34 (2000).
- Prado, F.E., J.A. Gonzalez, M. Gallardo, M. Moris, C. Boero and A. Kortsarz: Changes in soluble carbohydrates and invertase activity in *Chenopodium quinoa* developed for saline stress during germination. *Curr. Top. Phytochem.*, **14**, 1-5 (1995).
- Scandalios, J.G.: Oxygen stress and superoxide dismutase. *Plant Physiol.*, **101**, 712-726 (1993).
- Sen Gupta, A., G.A. Berkowitz and P.A. Pier: Maintenance of photosynthesis at low water potentials in wheat. *Plant Physiol.*, **89**, 1358-1365 (1989).
- Sen Gupta, A., R.P. Webb, A.S. Holaday and R.D. Allen: Over expression of superoxide dismutase protects plants from oxidative stress. *Plant Physiol.*, **103**, 1067-1073 (1993).
- Siddique, M.R.B., A. Hamid and M.S. Islam: Drought stress effects on water relations of wheat. *Bot. Bull. Acad. Sin.*, **41**, 35-39 (2000).
- Singh, K.P. and K. Singh: Stress physiological studies on seed germination and seedling growth of maize composites. *Acta. Bot. Indica*, **9**, 141-143 (1981a).
- Singh, K.P. and K. Singh: Stress physiological studies on seed germination and seedling growth of some wheat hybrids. *Ind. J. Plant Physiol.*, **25**, 180-186 (1981b).
- Singh P., P. Bhaglal and S.S. Bhullar: Differential levels of wheat germ agglutinin (WGA) in germinating embryos of different wheat cultivars in response to osmotic stress. *Plant Physiol. Biochem.*, **34**, 547-552 (1996).
- Soltani, A., S. Galeshi, E. Zeinali and N. Latifi: Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. *Seed Sci. Technol.*, **30**, 51-60 (2002).
- Sunderland, N.: Cell division and expansion in the growth of the leaf. *J. Exp. Bot.*, **11**, 68-80 (1960).
- Thomson, A.M., R.A. Brown, M.J. Rosenberg, R.C. Izaurrealde and V. Benson: Climate change impacts for the conterminous USA: An integrated assessment. Part 3. Dry land production of grain and forage crops. *Clim. Change*, **69**, 43-65 (2005).
- Tilki, Fahrettin and Huseyin Dirik: Seed germination of three provenances of *Pinus brutica* (Ten.) as influenced by stratification, temperature and water stress. *J. Environ. Biol.*, **28**, 133-136 (2007).

- Ungar, I.A.: Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). *Ame. J. Bot.*, **83**, 604-607 (1996).
- Uniyal, R.C. and A.R. Nautiyal: Seed germination and seedling extension growth in *Ougeinia delbergiodes* Benth. Under water and salinity stress. *New Forests*, **16**, 265-272 (1998).
- Vyas, S.P., S. Kathju, B.K. Garg and A.N. Lahiri: Activities of nitrate reductase and ammonia assimilating enzymes of moth bean under water stress. *Sci. Cult.*, **62**, 213-214 (1996).
- Willekens, H., W. Van Camp, M. Van Montagu, D. Inze, Jr. H. Sandermann and C. Langebartels: Ozone, sulphur dioxide and ultra violet-B have similar effects on mRNA accumulation of antioxidants genes in *Nicotiana plubaginifolia* (L.). *Plant Physiol.*, **106**, 1007-1014 (1994).
- Wilson, A.M.: Amylase synthesis and stability in crested wheat grass seeds at low water potentials. *Plant Physiol.*, **48**, 541-546 (1971).
- Woodel, S.R.J.: Salinity and seed germination patterns in coastal halophytes. *Vegetation*, **61**, 223-229 (1985).
- Yamazaki, J., A. Ohashi, Y. Hashimoto, E. Negishi, S. Kumagai, T. Kubo, T. Oikawa, E. Maruta and Y. Kamimura: Effect of high light and low temperature during harsh winter on needle photodamage of *Abies mariesii* growing at the forest limit on Mt. Norikura in Central Japan. *Plant Sci.*, **165**, 257-264 (2003).
- Zelitch, I. and S. Ochoa: Oxidation and reduction of glycolic and glyoxylic acids in plants. I glycolic acid oxidase. *J. Biol. Chem.*, **201**, 707-718 (1953).
- Zhang, J.X. and M.B. Kirkham: Drought stress induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol.*, **35**, 785-791 (1994).
- Zhao, F., S. Guo, H. Zhang and Y. Zhao: Expression of yeast SOD2 in transgenic rice results in increased salt tolerance. *Plant Sci.*, **170**, 216-224 (2006).