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Assessment of drought tolerance of selected wheat cultivars under laboratory condition

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A laboratory experiment was conducted during winter season of January to April, 2008 with five selected wheat varieties viz., K 9107, HD 2954, RAJ 4125, HUW 234, NW 2036. In order to assess the relative capability of drought tolerance various morpho-physiological parameters (water uptake by seeds, relative water content, fresh weight, dry weight , germination percentage, root length, shoot length, Tolerance index, %difference from growth, pH of seed leachate) were studied . From the results it was observed that RAJ 4125 variety were found to be mostly drought tolerant among the other experimental varieties having significant higher germination percentage, drought tolerance index value.

Key words: Drought, tolerance, wheat, cultivars, PEG, water deficit.

Introduction

Drought is one of the prevalent environmental conditions that cause adverse effects on the growth of plants. Water deficit more than other stresses limit the growth and productivity of crops. Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious (Pan *et al*., 2002). In rain fed agricultural conditions, water limitation may prove to be a critical constraint to primary productivity under future scenarios or more arid climates due to global environmental changes (Fischer *et al*., 2001). In meteorological terms, drought implies a relatively prolonged dry spell (absence of rains) resulting in moisture stress in the soil detrimental to crop growth especially in rain fed agriculture. The amount of water used by a crop is closely associated with photosynthetic activity, dry matter production and yield in many species (Tollenaar and Aguilera, 1992; Qing *et al*., 2001). However, the maximum photosynthetic

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potential of crops is seldom reached due to unfavorable environmental factors, including drought. Physiologists refer drought as water stress irrespective of length of period. Water deficit occurs in the plant whenever transpiration exceeds absorption. It may be due to reduced absorption of water from soil, excessive water loss or both. In other words, drought may be defined as the inadequacy of water availability including precipitation and soil moisture storage capacity and distribution during the lifecycle of the crop to restrict expression of its full genetic potentials (Sinha, 1986).

Wheat belongs to the family Graminae (Poaceae). Plant is annual herb, height up to 1-1.5 meter leaf blades linear, flat, auricled. Inflorescences erect spikes; spike lets solitary, sessile, laterally compressed, several flowered. Stamens 3, styles very short. Grain oblong, ventrally grooved. Indo-Gangetic plain is the best wheat producing region as the winter of this region is cool and the crop can grow for a period of 6 month. Areas having moist and warm climate, an annual rainfall range of 130-1000 mm, wheat is grown effectively. The best soil for wheat cultivation easily irrigatable and drainable loamy or clay-loamy soils.

Wheat is a worldwide cultivated grass from the Levant area of the Middle East. Globally, after maize, wheat is the second most produced food among the cereal crops. Wheat again is a staple food used to make flour for leavened, flat and steamed breads; cooking, pasta, noodles and couscous and for fermentation to make bear, alcohol, vodka or biofuel. The straw can be used as fodder for livestock, and as a construction material for roofing thatch.

PEG provides a means of quantifying the water stress that roots experience. The use of PEG-6000 for the experimental control of moisture stress in Petridis and pot culture studies has been considered very useful tool for the studying the effect of water stress for seed germination and seedling growth and consequently for evaluating the drought resistance character of plants (Deka and Baruah, 1998). It has been suggested to use PEG for imposing and studying the experimentally determinable moisture stress in crop plants under laboratory as well as under field conditions. Drought resistance is the result of numerous morphological, anatomical and physiological characters, which interact with maintenance of growth and developmental processes under edaphically and climatic conditions (Steponkus *et al*., 1980). The degree of limitation of yield by environmental stresses varies even among genotypes within a species (Wolfe *et al*., 1988; Aguilera *et al*., 1999). The present investigation was carried out to screen out best drought resistant wheat seed variety under laboratory condition and to study the morpho-physiological changes of wheat seedlings grown under drought stress.

Materials and methods

The experiment was conducted in the laboratory of the Department of Environmental Science, The University of Burdwan, Burdwan, West Bengal, India in 2008. Seeds that recently developed triticale varieties (K-9107, HD-2954, Raj-4125, HUW-234, and NW-2036) were used in the present study and are collected from Crop Research and Seed Multiplication Farm (CRSMF) OF The University of Burdwan, West Bengal. The experiment was carried out in randomized block design with three replications.

Artificial drought stress were created by preparing solution of PEG-6000 having osmotic potentials of -1.0 bars were arranged as described by Michel and Kaufmann (1973) by dissolving 78g in 1000ml of dist. water respectively. For the control condition distilled water were used. Screening of these cultivars was done by allowing them to grow for 6 days under PEG solution of -1.0 bars.

Seeds of different cultivars were surface sterilized with 0.1% $HgCl_2$ (w/v) for 2 min and presoaked in PEG solution (-1.0 bar) separately. 10 presoaked seeds of each cultivar were spread over a blotting paper in a petridish separately. The whole set was then placed under indoor laboratory conditions of bright diffused light, 70-80 % relative humidity and 25-30ºC temperature ranges. 10ml of PEG-6000 solution of -1.0 bars was added in each petridish in everyday. Surface sterilized seeds treated similarly with dist.water served as controls.

For morpho-physiological and growth studies of the five wheat cultivars the following methods were followed:

The relative water content was estimated according to Weatherly (1950) and was evaluated from the equation:

$$RWC = (FW-DW) / (TW-DW) \times 100$$

Where FW is the fresh weight of shoots, TW is the weight at all full turgor, measured after immersed in double dist. water for 4 hours in the light at room temperature and DW is the weight estimated after drying the shoots at 80 ± 1ºC in a hot air oven for 6 hours until a constant weight was achieved. Root and Shoot length (cm), fresh and dry weights of shoot and root of seedlings (g) were measured on the 8^{th} day. Dry weights were measured after drying samples at 80 ± 1ºC in hot air oven until a constant weight is achieved. Tolerance Index was measured with dividing of dry weight of seedlings in stress with dry weight of seedlings in control (Maiti *et al*., 1994).

Tolerance Index (TI) = Dry weight of seedlings in stress (g)/Dry weight of seedlings in Control (g)

Water uptake by seeds were also performed by 1 g weight of each seeds subjected to treatment with osmotic solution of -1.0 bar PEG and predetermined time 24 hours, washed with dist. water, blotted to dry immediately and weighed. Water uptake by seeds was calculated by using the following formula:

$$\text{Water Uptake} = W_2 - W_1 / W_1 \times 100$$

Germination percentage and % difference from control was also calculated (Mhatre and Chaphekar, 1982) at every 24 hours interval for 3 days. Germination percentage was calculated on the total number of germinated seeds per treatment after 3 days of incubation and percentage difference from control (% DFC) for germination was calculated from the following formula:

$$\% \text{ DFC} = \frac{\% \text{ germination of control} - \% \text{ germination of test sol} \times 100}{\% \text{ germination of control}}$$

Finally *p*H of the leachates of the different wheat variety was determined by using digital *p*H meter (Systronic Model 335). All the results were analyzed statistically through one way ANOVA analysis and interpreted after Panse and Sukhatme (1985).

Results and discussion

Germination percentage declined progressively under stressed condition than the controlled. NW 2036 showed 100 and 98.33 and RAJ 4125 showed 98.75 and 94.583 the highest germination percentage under both controlled and stressed conditions respectively. HUW 234 has the lowest result 95.416 and 88.333 in controlled and stressed conditions (Table 1).

The highest shoot length was 10.365cm observed in NW 2036 and lowest was 6.152cm in HUW 234 in case of controlled; on the other hand in stressed condition the highest shoot length was 18.06cm observed in HD 2954 and lowest was 3.647cm again in HUW 234. In case of root length highest result showed again by NW 2036 i.e. 11.075cm and lowest was HD 2954 i.e. 5.468cm in case of controlled and on the other hand in case of stressed highest root length observed in NW 2036 that was 9.116cm and lowest was in HUW 234 that was 2.981cm. Whereas RAJ 4125 showed a steady result in both conditions (Table 1).

Table 1. Germination rate and growth attributes of wheat cultivars under laboratory condition.

Varieties	Germination percentage (%)		Shoot length(cm)		Root length(cm)	
	Control	Stress	Control	Stress	Control	Stress
K 9107	99.583	92.916	7.447	6.122	8.86	7.379
HD 2954	99.166	92.916	6.892	18.06	5.468	6.191
RAJ 4125	98.75	94.583	7.409	7.291	6.781	6.729
HUW 234	95.416	88.333	6.152	3.647	7.145	2.981
NW 2036	100	98.333	10.365	8.546	11.075	9.116
SEM (±)	0.795	2.524	0.294	0.054	0.183	0.465
CD at 5%	3.734	6.653	2.271	2.974	1.794	2.857
CV (%)	0.904	1.692	7.086	11.595	5.445	10.531

The highest water uptake of seeds were 47.333 and 43 found in RAJ 4125 in both controlled and stressed conditions and minimum was 46 found in HUW 234 in case of controlled and 38.333 in K 9107 and HD 2954 in case of stressed condition (Table 2).

RWC of leaves of all the varieties decreased significantly over control in our present investigation. Highest RWC showed by RAJ 4125 that was 98.35 in case of controlled and again RAJ 4125 and NW 2036 that were 95.253 and 95.783 in case of stressed condition respectively (Table 2).

Table 2. Morpho-physiological attributes of wheat cultivars under laboratory condition.

Varieties	Water uptake by seeds		Relative water content	
	Control	Stress	Control	Stress
K 9107	47.333	38.333	91.497	86.727
HD 2954	46.333	38.333	87.803	85.667
RAJ4125	47.333	43	98.35	95.253
HUW 234	46	42.667	94.523	90.73
NW 2036	46.667	42	97.93	95.783
SEM (±)	0.269	0.357	0.24	0.491
CD at 5%	2.17	2.503	2.053	2.935
CV (%)	1.114	1.463	0.521	0.772

In this experiment NW 2036 have highest fresh and dry weight in both controlled and stressed conditions. In case of control highest fresh weight was 0.471g and dry weight was 0.072g whereas in case of stress result was 0.349g in NW 2036 and 0.082g in K 9107g respectively. On the other hand HUW 234 showed lowest results in both cases (Table 3).

Table 3. Morpho-physiological attributes of wheat cultivars under laboratory Condition.

Variety	Fresh Weight(g)		Dry Weight(g)	
	Control	Stress	Control	Stress
K 9107	0.416	0.282	0.063	0.082
HD 2954	0.283	0.208	0.043	0.033
RAJ 4125	0.26	0.19	0.051	0.055
HUW 234	0.339	0.15	0.05	0.023
NW 2036	0.471	0.349	0.072	0.066
SEM (±)	0.015	0.011	0.002	0.017
CD at 5%	0.512	0.433	0.203	0.543
CV (%)	34.569	43.814	87.345	280.708

The maximum pH value found in RAJ 4125, that was 6.45 and minimum was 6.187 found in K 9107. Water absorption rate, respiratory rate and storage reserve transformation rate of wheat seeds also increased with increased pH values (Table 4).

In this investigation highest % DFC value was 9.514 and found in HUW 234 and lowest was 5.585 found in RAJ 4125 (Table 4).

Maximum value of drought tolerance index was 1.237 found in RAJ 4125 that was far greater than other cultivars. On the other hand lowest tolerance index was 0.51 found in HUW 234. So, RAJ 4125 recognized as tolerant and similarly HUW 234 considered as most susceptible cultivar among different varieties which was further suspected by % DFC values (Table 4).

Table 4. pH of the leachate, Percentage difference from control and Tolerance index attributes of wheat cultivars under laboratory condition.

Variety	pH of the Leachate	% Difference from control	Tolerance index
K 9107	6.187	7.778	0.766
HD 2954	6.293	6.725	0.789
RAJ 4125	6.45	5.585	1.237
HUW 234	6.25	9.514	0.51
NW 2036	6.237	1.667	0.92
SEM (±)	0.006	3.93	0.096
CD at 5%	0.339	8.3	1.3
CV (%)	1.287	31.7	36.772

Polyethylene glycol (PEG) decreases the available water required for seed germination and plant growth. The inhibitory effect of PEG on seed germination of different plants was recorded by many investigators (Heikal and

Shaddad, 1982 on cotton, pea and wheat, Kuhad *et al*., 1987 on *Pennisetum americannum*). Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought stimulator (Ashraf *et al*., 1996; Turhan, 1997). In the present investigation PEG-6000 was used to create the osmotic stress, as most of the researchers (Smok *et al*., 1993; Hu and Jones, 2004) utilized it for the development of water deficit environment in growth chamber studies.

Seed germination is first critical and the most sensitive stage in the life cycle of plants (Ashraf & Mehmood, 1990) and the seeds exposed to unfavorable environmental conditions like water stress may have to compromise the seedlings establishment (Albuquerque & Carvalho, 2003). In the present investigation the reduction in germination might be due to the less availability of free water to the seeds. Germination percentage and germination rate generally decrease as soil water potential decreases (Murillo-Amador *et al.,* 2002; Song *et al.,* 2005; Sosa *et a.,l* 2005), by drought. Therefore, it seems that water potential exerts major influence on seed germination (Bewley and Black, 1994; Larson and Kiemnec, 1997) and osmotic stress delayed the emergence of the radicle and further development of the seedling, similar to the report of Maliwal and Paliwal (1970). The retardation of germination by chemicals may be due to their osmotic or ionic effects or a combination of both (Greenway and Munns, 1980).

Seedling growth as dry mass, plant height and root length indicate that seedling growth is a reliable and efficient procedure for screening for moisture stress tolerance (Shamim Ahmad *et al*., 2009 for sunflower; Ashraf *et al*., 1996 for wheat)

In our present investigation there was a declining pattern in the water uptake by seeds in comparison to control among five wheat cultivars which may be attributed towards the external water potential which decreased due to reduce diffusivity of seed coat to water at lower water potential. PEG lowers the osmotic potential of the external medium and reduces water availability for germinating seeds.

Physiological parameters such as relative water content (RWC) is also very responsive to drought stress and have been shown to correlate well with drought tolerance (Jamaux *et al*., 1997; Altinkut *et al*., 2001; Colom and Vazzana, 2003).The relative water content of the leaves has been proposed as a better indicator of water stress than other growth/biochemical parameters of the plant (Sinclair and Ludlow, 1985). The RWC is usually higher in plants, which are adapted to dry conditions, and similar observations had earlier been recorded by Carter and Paterson 1985 in soybean. The reducing trend of relative water content for all the five wheat varieties may be attributed towards reduction in the external water potential. At particular water potential higher

RWC (%) is an indicator of drought tolerance through osmoregulation with higher RWC under higher external osmotic potentials. For the tolerant variety, an active accumulation of solutes (osmoregulation) takes place. This is the only adaptive and positive response beneficial to the plant under water stress condition, (Turner, 1986). Osmoregulation enables the plant to maintain high turgor pressure and as well to survive under stress condition. The osmotic potential is considered an important selection criterion for rice. In the present investigation the highest RWC % was found in RAJ 4125 and NW 2036 in both controlled and stressed condition which further supports their response towards drought tolerance.

In our present investigation water deficit adversely affected vegetative growth as indicated by changes in dry weight. The results are comparable to those of Rahman *et al*. (2000). David and Park (1979) had earlier observed decreases in dry weight of *Phaseolus vulgaris* under drought conditions, which is similar to the results of this present study. Although plant height and dry matter decreased with the increase in water stress created by PEG 6000 but different cultivars showed different performances under water stress environment (Ashraf *et al*., 1996). The reducing trend of the shoot length and biomass of wheat cultivars may be attributed towards the drought stress. On the other hand variety RAJ 4125 and NW 2036 showed higher biomass and shoot lengths was found to be a reliable indicator of drought tolerance in wheat in the present investigation.

In our present investigation dry matter tolerance index was decreased significantly with the increase in PEG concentration. Highest value of drought tolerance index was found in RAJ 4125 that was far greater than other cultivars in experimental condition. Wheat variety RAJ 4125 and NW 2036 reflect greater amount of tolerance towards drought stress through their lower % DFC value and variety K 9107 and HUW 234 showed higher % DFC are susceptible to environmental stress condition.

Optimal germination PH is between 6 and 7.5. Wheat seeds germinate abnormally at decreased PH values (Zeng QL, Huang XH, Zhou Q, 2005). Therefore, on the basis of observed PH in the present investigation RAJ 4125 found to be the best fitted wheat seed cultivar among the all varieties. The present study was conducted with the objectives to determine the response of wheat varieties to drought stress at germination and seedling stages under controlled conditions and to evaluate germination and seedling growth as screening criteria for drought tolerance in wheat. The rate and degree of seedling establishment are extremely important factors in determine both yield and time of maturity (Rauf *et al*., 2007). Wheat crops often experience water deficit and heat stress during grain growth and development, which limit productivity. High temperature during the terminal grain filling period (+35ºC) and post-anthesis water deficit conditions in irrigated wheat, influence crop growth and yield. In this present experiment the wheat variety RAJ 4125 reflects greater amount of tolerance towards drought stress through their lower % DFC value, maximum value of drought tolerance index and relative water content value. From these results we can conclude that RAJ 4125 may be distributed among the Farmers in the drier part of the West Bengal, particularly lateritic belt for wheat production where water deficiency is a common problem of agricultural field.

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