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Influence of Seed Hardening Techniques on Vigor, Growth and Yield of Wheat under Drought Conditions

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Abstract

Exploring ways to improve stand establishment and crop productivity under abiotic stresses like drought is important. Two years experiments were conducted at University of Agriculture, Peshawar-Pakistan to examine the efficacy of six pre-sowing seed hardening agents. Seeds of wheat cultivar Uqab-2000 were hardened in six different chemicals of various concentration viz; PEG-8000 (10%), $CaCl_2$ (4%), KNO_3, (3%), Mannital (4%), NaCl (5%), Na_2SO_4 (2%) along with water soaking and dry seeds as control for 24 hours and drying back to original moisture content at room temperature. The soaking and drying of seeds was repeated twice for 12 hours. The results showed that pre-sowing hardening of seed with PEG-8000, $CaCl_2$ and KNO_3 gave higher germination, decreased days to 50% germination, increased shoot length, root length, seedling fresh and dry weight in laboratory experiment as compared with other hardening and control treatment. Under field conditions, maximum plant height (93.53cm), spikelet's spike^{-1} (17.16), grains spike^{-1} (50.82), 1000 grain weight (39.97 g), grain yield (3.43 tha^{-1}) and maximum harvest index (32.5%) were observed in PEG-8000 hardened seed than control treatment (2.82 tha^{-1}). Seed hardened in PEG-8000, $CaCl_2$ and KNO_3 gave 30% increase in grain yield as compared to Mannital, NaCl and Na_2SO_4 which gave 15% increase in grain yield over control treatment.

Keywords: Seed enhancement; Attributes; Growth; Yield improvement: Environment

1. Introduction

Wheat (Triticum aestivum L.) is an important food crop of Pakistan and mostly cultivated in dryland areas, where rainfall is scarce. Extreme temperatures, soil crusting, low rainfall and salinity may individually or in combination adversely affect the germination and stand establishment of the crop and reduces grain yield (Basra et al., 2002: Anguish et al., 2001). Efforts made to maximize yield were hampered by the adverse effect of abiotic stress such as salinity and drought. These effects cause huge losses due to low yield and failure of the crop to establish in dryland areas (Khan, 1992). Pre-sowing hardening technique has been used as an alternate approach to overcome the ailing effects of abiotic stresses in agricultural production because of its low cost and risk. It improves seed emergence and growth of crops (Pill and Necker, 2001). Pre-sowing hardening technique is a repeated soaking and control seed hydration in solution containing organic or inorganic solutes followed by redrying that allows pre-germinative metabolic activities but prevent radical emergence (Basra et al., 2005). The hardening treatment proved to be better for vigor enhancement than the traditional soaking (Manjunath and Dhanoji, 2011). Seed hardening treatments enhances seeds vigor by protecting structure of the plasma membrane against injury during stress environment (Bewley and Black, 1994; JunMin *et al*., 2000). Pre-soaking seeds with optimal concentration of phyto-hormones enhance their germination, growth and yield under stress condition by increasing nutrient reserves mobilization through increased physiological activities and root proliferation (Ozturk et al.,1993).

Previous studies have also shown that pre-sowing seed treatment in various concentrations of Indole acetic acid (Gulnaz et al., 1999), Gibberellic acid (Shah 2007) and Indole Butyric acid (Strader et al., 2011) may promote or inhibit seedling growth. Therefore, this study was designed to evaluate the effect of seed hardening techniques on vigor, growth and yield attributes of wheat under rainfed environment.

2. Materials and Methods

2.1 Experimental Site and Background

Field and laboratory trials were carried out at the Agronomy Research Farm, University of Agriculture, during 2010-11 and 2011-12. The experimental farm is located at 34.01° N latitude, 71.35° E longitude, at an altitude of 350 m above sea level. Peshawar is located about 1600 km north of the Indian Ocean and has continental type of climate (Fig.1). Soil is clay loam, low in organic matter (0.87 %), phosphorus (6.57 mg kg^{-1}), potassium (121 mg kg^{-1}), alkaline (pH 8.2) and is calcareous in nature. Seeds of wheat cultivar Uqab-2000 obtained from the Research Farm, Agriculture University, Peshawar, were surface sterilized in 10% sodium hypochlorite solution for 10 minutes, then rinsed with sterilized water and air-dried at room temperature closely to original moisture level. Before planting the experiment, various concentrations of the growth substances prepared in the laboratory were transferred into 500 mL conical flasks which were clearly labeled according to the concentration of the growth substances to be used for hardening purpose. 250 g seed was soaked in solution of six different chemicals viz. PEG-8000, Mannital, KNO_3, NaCl, $CaCl_2$ and Na_2SO_4, each in concentrations @ 10%, 4%, 3%, 5%, 4% and 2% for 24 hours before sowing, dried back and cycle was repeated twice for 12 hours. There were two controls: water soaking and dry seed/control treatment which were not pre-soaked before sowing. Seed were subjected to six pre-sowing hardening treatment namely (T_1) Control/Untreated seed (T_2) hydration for 24 hours in water (T_3) hydration with PEG-8000 (10%) solution for 24 hours

(T_4) hydration with 4 percent $CaCl_2$ for 24 hours (T_5) hydration with 4 percent Mannital for 24 hours (T_6) hydration in 3 percent KNO_3 for 24 hours (T_7) hydration in 5 percent NaCl for 24 hours (T_8) hydration in 2 percent Na_2SO_4 for 24 hours. The solution to seed ratio was **1:2.5** for all the treatments. These seeds were further used for vigor determination in laboratory and for growth and yield attributes under field conditions. In laboratory experiment, 25 seeds each of control and treated seeds were sown in plastic trays filled with moist sand, replicated three times and were placed in growth chamber at 25℃ temperature in a completely randomized design, whereas, a randomized complete block design (RCBD) with three

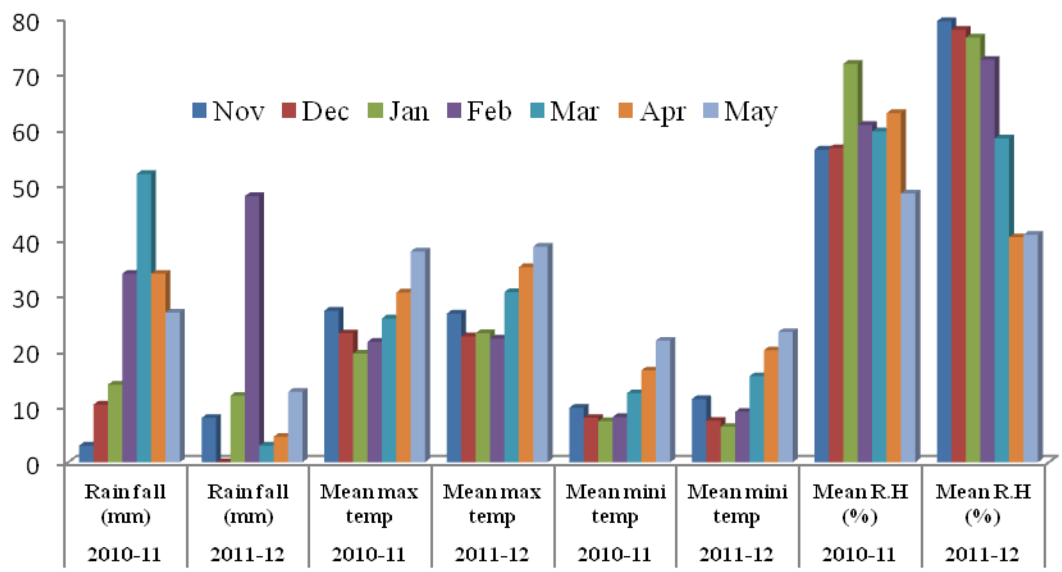

Fig. 1: Monthly maximum, minimum temperature (°C), relative humidity (%) and rain fall (mm) during growing season 2010-11 and 2011-12.

replications was used for field experimentation. A net plot size of 7.5 m^2 (1.5 m x 5 m) having 5 rows of 30 cm apart and plant to plant 4 cm was used. A basal dose of 120 and 60 kg ha^{-1} N and P_2O_5 were applied using urea (46% N) and diammonium phosphate (18% N, P 36%) as fertilizer source. Full dose of phosphorus and half of N were applied at sowing, while remaining half of N was applied in two equal splits each at tillering and jointing stage. Hoeing, weeding and other cultural practices were done as and when required. The experiments were harvested during 2nd week of May each year.

2.2 Laboratory Observations

2.2.1 Germination Test

Germination potential of controlled and treated seeds was estimated in accordance with (ISTA, 2009). To test seed germination and seedling vigor, four replicates of 25 seeds were germinated in 12cm diameter petridishes. The petridishes were covered with lids and placed in incubator at 25°C. A seed was considered to have germinated when coleoptile and radical lengths have reached 2 mm. Counts of germinating seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was achieved. The time to reach 50% germination (T_{50}) of final germination was calculated according to the following formula of Coolbear et al. (1984):

$$T_{50} = t_i + \frac{\left[\frac{N}{2} - n_i\right](t_j - t_i)}{n_j - n_i}$$

Where N is the final number of germination and ni, nj cumulative number of seeds germinated by adjacent counts at time ti and tj when ni < N/2 < nj.

2.2.2 Germination Index (GI): was calculated according to ISTA (2009) formula

$$G1 = \frac{\text{No. of germinated seeds at first count}}{\text{Days of first count}} + - - + \frac{\text{No. of germinated seeds at final count}}{\text{Days of final count}}$$

2.2.3 Germination Energy (GE)

Germination energy (%) was recorded on the 4th day of the experiment. It is the percentage of germinated seeds 4 d after the start of the experiment relative to the total number of seeds tested (Ruan *et al*. 2002).

2.2.4 Seedling emergence

Control and treated seeds were sown in plastic trays (25 in each) having moist sand, replicated three times and were placed in growth chamber at 25 ℃ temperature in completely randomized design. Emergence was recorded daily according to ISTA rules (2009). Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MET = \Sigma Dn / \Sigma n$$

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of emergence. The plants were harvested 12 days after planting and seedling vigor data were recorded according to ISTA rules (2009).

2.2.5 Measurement of Root and Shoot Length

Root and shoot length of all 25 seedlings were measured after 12 days of experiment. It was measured with a measuring scale and expressed in centimeters. These seedlings were kept in brown paper and weighed the fresh weight first and were died in oven at 70 ℃ for 48 hours to record the dry weight. These were measured with electronic balance and expressed in grams.

3. Field Observations

3.1 Agronomic and Yield Component Traits

At maturity the height of randomly selected ten plants was measured with a meter rod from base to tip of the spike and average was worked out. Spikelet's spike^{-1} data was recorded by counting the number of spikelets in ten randomly selected spikes. Number of grains spike^{-1}

was counted manually. Grains from 10 spikes were collected and then calculated as average number of grains spike^{-1}. Four central rows of each plot were harvested carefully, tied into bundles, sundried, threshed and cleaned to record grain yield (kg ha^{-1}).Thousand grains from each treatment were taken at random and weighed in grams. The harvest index (%) was calculated after harvest by using the formula:

$$\text{Harvest index (HI)} = (\text{G.Y}/\text{B.Y})*100$$

3.2 Statistical Analysis

Data on different parameters were subjected to analysis of variance (ANOVA) according to the methods described for completely randomized design (CRD) and randomized complete block design (RCBD) combined over the years and means between treatments were compared using LSD test at 5% probability level (Steel & Torrie, 1997).

4. Results and Discussion

4.1 Germination (%)

The data revealed that all hardening treatment significantly affected the germination and its related attributes. Majority of chemical agents along water soaking gave faster and earlier germination at 24 h hardening except NaCl (5%), Na_2SO_4 (2%) and control treatment (Table 1).

Table 1. Mean performance of pre-sowing hardening treatments on germination behavior of wheat (combined across 2010 and 2012 seasons).

Treatments	Germination (%)	T_{50} (days)	MET (days)	Ger. Index (G.I)	G. Energy (%)
Control	51 ik	2.75 a	4.3 a	17.0 d	35.0 d
Water soaking	82 ab	1.40 cd	2.3 cd	33.5 b	91.3 ab
PEG 8000(10%)	89 a	1.00 de	2.4 cd	37.2 a	95.0 a
Mannital(4%)	61 di	1.55 c	2.8 d	23.0 c	73.0 b
KNO_3 (3%)	83 ab	1.25 cd	2.6 cd	32.3 bc	70.0 bc
NaCl (5%)	55 e-k	2.34 ab	3.4 b	21.2 cd	62.3 d
$CaCl_2$ (4%)	87 a	1.15 d	2.3 cd	35.4 ab	90.0 a
Na_2SO_4 (2%)	68 cd	2.30 ab	3.2 b	22.7 c	67.0 cd

*Means of the same category followed by different letters are significantly different at 0.05 level of probability using LSD test T_{50}= Days to 50% germination; MET = Mean emergence time GI = Germination index; GE = Energy of germination

Maximum germination percentage (89%) was observed in seeds hardened for 24 h in PEG-8000 (10%) followed by $CaCl_2$ (4%) and KNO_3 (3%) treated seed. The reason for higher germination % and rate may be due to greater hydration of colloids, higher viscosity and elasticity of protoplasm, offer an increase in bound water content, lower water deficit and increased metabolic activity (Maitra et al., 1999). The improvement in germination by PEG-8000 (10%), $CaCl_2$ (4%) and KNO_3 (3%) hardened seeds may be attributed to stimulation of hydrolytic enzyme activity known to be induced by these chemical agents. While in emergence test (Table 1) the results showed that hardening treatments and water soaking significantly reduced germination time and improved the emergence rate and seedling vigor (Bray et al., 1989). This indicates that hardening treatment and water soaking not only improves the performance of low vigor seeds but can also invigorate and induce early, synchronized and healthier crop stand. Hardening for 24 hours improved germination rate, time and also enhanced the vigor attributes. Greater efficiency of osmo-hardening with $CaCl_2$ and KCl is possibly related to the osmotic advantage that both K^+ and Ca^{2+} have an improving cell water status and also act as cofactors in the activities of numerous enzymes (Joseph and Nair 1989), most of which are active when reserve metabolization and radical protrusion were in progress. Lowest germination (%) was recorded in NaCl (5%) and dry seed/control treatment (Table 1). The decrease in germination (%) by NaCl might be due to accumulation of salts in tissue, which cause toxicity (Smith and Cobb 1991). Maximum emergence time was observed in NaCl, Na_2S0_4, and Mannital hardened seed, which resulted in delayed and scattered germination (Fig. 2). Decrease in T_{50} by water soaking may be due to faster production of germinative metabolites and better genetic repair i.e., earlier and faster

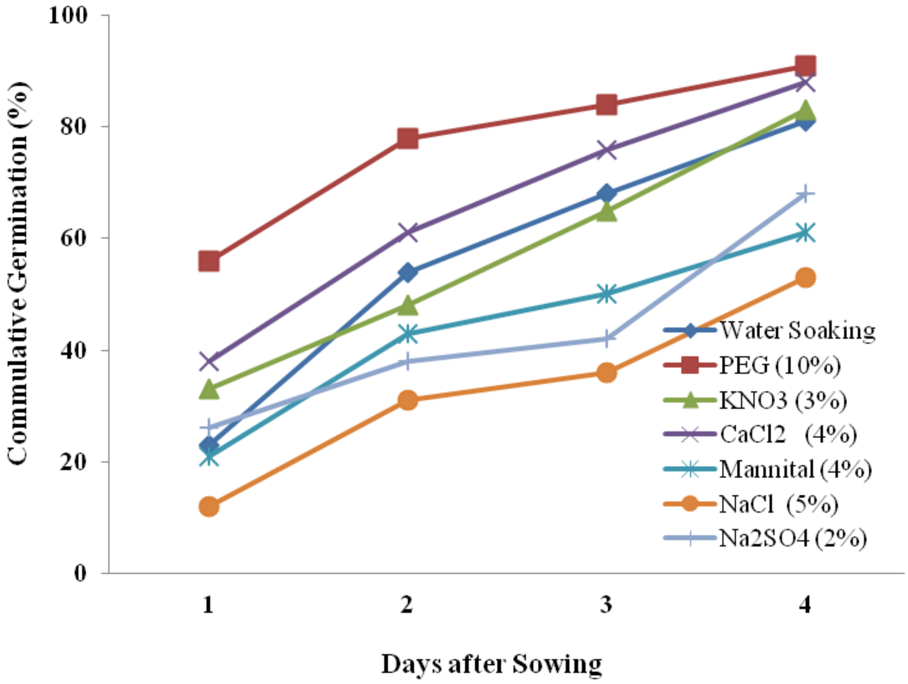

Fig. 2: Effect of pre-sowing hardening treatments on the germination of wheat seeds.

synthesis of DNA, RNA and protein (Bray *et al*., 1989). These results are in line with the findings of Lee et al., (1998) and Basra et al. (2002) who reported that hardening treatment reduces the T_{50} in wheat and rice seed. The decrease in E_{50} by various hardening chemicals might be due to controlled hydration, which results in better structural and genetic repair (Bray et al., 1989). Germination index and energy of germination data support the trend of other germination attributes. Maximum invigoration was observed in pre-germinated seeds hardened for 24 h in PEG-8000 (10%), $CaCl_2$ (4%), KNO_3 (3%) and water soaking. Majority of pre-sowing hardening chemicals resulted in vigor enhancement as compared with control. NaCl and Na_2SO_4 hardening treatment could not invigorate the wheat seeds rather these reduced the vigor and prolong the germination time.

4.2 Seedling Vigor

Data regarding seedlings vigor revealed that hardening and water soaking for 24 h gave earlier and rapid emergence (Table 2). Most seed hardening chemicals significantly affected all vigor traits as compared to control treatment. Seedling vigor traits improved by 25% by hardening treatments, which was 12% more than control treatment. Significant increase in lengths of shoot and root were observed in 24 h hardening with PEG-8000, $CaCl_2$ and KNO_3 which was statistically at par to water soaked seeds. A half-way increase in shoot/root length was observed in Mannital (4%), NaCl (5%), and Na_2SO_4 (2%) hardened seed as compared to dry seed. This is in line with the findings of Gulnaz et al., (1999) who stated that both quantitative and qualitative responses of plants to different hormones/chemicals may differ considerably at different plant growth stage. Pre-sowing hardening treatment gave maximum fresh and dry seedling weight which is similar to water soaking treatment. Control treatment

Table 2. Mean performance of pre-sowing hardening treatments on seedling vigor of wheat (combined across 2010 and 2012 seasons).

Treatments	S. L (cm)	R.L (cm)	S.f.wt (mg)	S.d.wt (mg)	R.f.wt (mg)	R.d.wt (mg)
Control	6.8 c	5.5 c	18.5 d	5.5 e	6.7 e	2.6 c
Water soaking	10.1 a	8.9 a	25.6 b	11.2 b	14.5 c	4.4 a
PEG 8000(10%)	13.6 a	11.4 a	30.4 a	14.4 a	20.2 a	5.8 a
Mannital(4%)	7.7 c	6.3 b	17.4 d	8.2 cd	9.8 d	2.0 c
KNO_3 (3%)	10.4 ab	8.8 a	23.5 c	9.4 c	17.6 cd	3.3 b
NaCl (5%)	7.8 c	5.8 b	13.2 de	6.8 d	5.8 e	2.0 c
$CaCl_2$ (4%)	12.2 a	10.2 a	24.7 bc	10.4 b	12.5 a	4.7 a
Na_2SO_4 (2%)	8.4 bc	6.2 b	15.4 d	8.3 cd	8.3 d	2.0 c

*Means of the same category followed by different letters are significantly different at 0.05 level of probability using LSD test S.L= Shoot Length; R.L= Root Length; S.f.wt= Shoot fresh weight; R.f.wt= Root fresh weight; S.d.wt = Shoot dry weight; R.d.wt = Root dryweight

gave minimum fresh dry weight.This indicates that during hardening, seeds were simultaneously subjected to the processes of repair and deterioration and force between the two determinants of success or failure of the treatment. Improved seedling dry weight by hardened seeds is attributed to more synchronize germination, which resulted in early stand establishment. Higher shoot fresh weight and length in seedlings from 24 h hardened seed is an indication of early emergence and more rapid rate of emergence of wheat seed (Table 2). Earlier work of (Shah 2007) support the findings of current research in which hydro-priming and hardening resulted in higher fresh and dry weight of seedlings. Longest shoots were noted in 24 h seed hardening. The findings of present study are in agreement with the results of Kaya et al., (2006) and Basra et al., (2006) who observed that hardening and hydro-primed seeds of sunflower and wheat could germinate faster and produced longer seedling under drought/salinity stress as compared with untreated seeds. Hardening of wheat seeds in NaCl and Na_2SO_4 could not strengthen the wheat seeds rather these reduced the vitality and increase emergence time.

4.3 Agronomic and yield related traits

Data regarding agronomic and yield traits showed that Pre-sowing hardening technique had profound effects on the agronomic and yield related traits of wheat (Table 3). Seed hardening for 24 hours with PEG-8000, $CaCl_2$ and KNO_3 improved plant height, spikelet's spike^{-1}, grains spike^{-1},1000-kernel weight, grain yield (kg ha^{-1}) and harvest index (HI) as compared to other hardening and control treatment. Maximum plant height of 93.53cm was recorded in PEG-8000 hardened seed followed by $CaCl_2$ and KNO_3, where as lower plant height was recorded in control treatment. The increase in plant height with seed hardening might be due to cell enlargement and improvement in normal cell division. Similar increase in plant height and yield in sunflower was observed by Pawar et al., (2003). Who reported that these chemicals activate the synthesis of proteins and soluble sugars in the first phase of germination which have advantages for subsequent growth phases (Jyotsna and Srivastava,

Table 3. Mean performance of pre-sowing hardening treatments on wheat growth and yield under drought condition (combined across 2010 and 2012 seasons).

Treatments	Plant height (cm)	Spikelet's/spike	Grains/spike	1000-grain(wt) (g)	Grain yield (tha^{-1})	H.Index (%)
Control	75.51 e	13.55e	33.67e	31.89d	2.63 e	28.51cd
Water soaking	81.29ab	15.41b-d	38.59b	38.68a	3.10 ab	30.91b
PEG 8000(10%)	83.53 a	16.16 a	42.82a	39.97a	3.23 a	32.53 a
Mannital(4%)	80.22bc	15.10ce	38.29bc	36.78ab	3.05 bc	31.06 ab
KNO_3 (3%)	83.38 a	15.93ab	41.09a	37.98a	3.17 ab	30.10 b
NaCl (5%)	76.69 de	14.73e	36.31cd	34.58cd	2.68 de	28.35 c
$CaCl_2$ (4%)	82.64 a	15.76a-c	40.53a	38.70a	3.20 ab	31.31ab
Na_2SO_4 (2%)	78.44 cd	14.57e	36.53b-d	35.19cd	2.98 cd	30.54 bc

*Means of the same category followed by different letters are significantly different at 0.05 level of probability using LSD test

1998). Increased number of spikelet's spike^{-1}, grains spike^{-1} was recorded for 24 h hardening treatment.Similarly, maximum grain yield and harvest index (HI) was also recorded for PEG-8000 hardening treatment and were statistically at par with water soaking (Table 3). The improvement in growth and yield attributes by hardening techniques indicates mode of action vary for the chemicals studied. Similar results were reported by Dighe et al., (1983) in wheat. The increase in the yield by seed hardening with PEG-8000, KNO_3 and $CaCl_2$ may be due to improvement in yield components mainly 1000 seeds weight, seed yield plant^{-1} and harvest index. Lower yield and lower mean value for yield attributes were observed in control treatment. These results are in agreement with Manjunath and Dhanoji, (2011) who stated that treating wheat seeds with 0.25% $CaCl_2$ or 2.5% KCl increased the grain yield compare to control. The increase in grain yield with pre sowing treatments was due to the expansion of leaves, which resulted in higher photosynthesis, assimilation and ultimately higher production of total dry matter. Misra and Dwibedi (1980) reported 37 % increase in grain yield of wheat due to pre-sowing techniques. Haris et al., (1999) observed 15 % increase in grain yield by seed hardening and stated that success of hardening techniques depends on type of cultivar, osmotic potential solution, temperature, seed vigor, rate of seed re-drying and conditions during seed storage.

5. Conclusion

The results of the present investigations lead us to the conclusion that seed hardening with PEG-8000, $CaCl_2$ and KNO_3 for 24 h showed maximum invigoration for improving vigor, growth and yield in wheat under drought stress. These hardening chemicals could be effectively used in wheat crop for the amelioration of drought under rainfed environment.

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