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Callogenesis and Organogenesis Response of Wheat Cultivars under Sodium Chloride Salt Stress

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Abstract: The study was conducted at the Agricultural Biotechnology Research Labs, National Agricultural Research Centre, Islamabad to ascertain varietal differences between four wheat varieties (Arz, Pak-81, LU-26 and Pavon); with respect to their tolerance to sodium chloride (NaCl) stress indicated by callogenesis and organogenesis. Murashige and Skoog (MS) modified medium supplemented with 50, 100, 150 and 200 (mM) sodium chloride salt was used for tissue culture. Results clearly indicate striking differences among different wheat varieties in callus induction and regeneration under salinity stress. LU-26 was the most tolerant cultivar followed by Pavon, Pak-81 and Arz. The findings can help in breeding for salt tolerance in wheat.

Key words: Wheat, *Triticum aestivum* (L.), cultivars, salt stress, callogenesis, organogenesis, regeneration

INTRODUCTION

Of the world's available land area (14 billion ha), only around 3.2 billion ha are arable and 2% of this arable land is subjected to excess salinity, a problem both in arid regions and in some areas near the ocean. Another 25% of arable land suffers from excess rainfall and, therefore, soil acidity. As hydrogen ion concentrations build up in the soils, other more useful cations are leached from the soil particles; all these cause interference with mineral availability and uptake (Nabors and Dykes, 1985).

Salinity has created an alarming situation in Pakistan. The poor crop growth in saline soils is thought to be due to such causes as imbalance in mineral nutrition, reduction in water uptake and/or direct salts toxicity to plants. Agricultural crops and their varieties differ considerably with regard to salt tolerance at various growth stages; germination and seedling being generally the most critical ones (Brown and Hayward, 1956). Soil salinity at the time of seedling and emergence of rice resulted in greater decrease in grain yield than a similar level of salinity induced when the plants were six weeks old. The reduction in yield was attributed to a decrease in germination percentage.

Younis and Hatata (1971) reported considerable evidence that increase in salinity reduces seed germination and growth in many crops including wheat. Similarly, reduced crop yield and delayed flowering were reported in barley and corn by Hassan *et al.* (1970). In selecting wheat varieties for saline soils, particular attention should be paid to salt tolerance during emergence because poor crops frequently result from failure to obtain a satisfactory stand (Kapp, 1947). Seedlings of *Triticum aestivum* cultivars Lyallpur-73, Pak-81 and LU 26-S were exposed to varying concentrations of NaCl for 8 days by Hanif and Davies (1998). Increasing concentrations of NaCl reduced the size of apical meristem of wheat cultivars and caused the most distal root hair to form closer to the root tip. They also investigated in another study that an 8 days exposure to NaCl salt reduced the length of root apical meristem in Scale cereal cultivars to a greater extent than *Triticum aestivum* cultivars (Chinese Spring).

Farrukh (2002a) also subjected one month old calluses of wheat cultivars LU-26 and Potohar to different salt concentrations (100 or 200 mol m^{-3}) in MS liquid medium to assess the effects of salinity stress on the growth. He found that growth rate of calluses decreased with increasing salts concentrations. Callus induction in Potohar variety showed higher growth rate reduction than LU-26.

Embryogenic calli of three varieties of wheat viz., Lyallpur-73, Pak-81 and Hyderabad-88 initiated and maintained on Murashige and Skoog (MS) basal medium supplemented with 3 mg L^{-1} 2, 4-D (2, 4- dichlorophenoxy acetic acid) were stressed with varying levels of sodium chloride (NaCl) at 50, 100 and 150 mM (Tahir *et al.*, 2002). Resistance in the three varieties was in the order of Hyderabad-81 greater than Pak-81 greater than Lyallpur-73 (Tahir *et al.*, 2002). Tissue culture can increase the stress tolerance ability of plants cell suspension or callus (contains perhaps 50,000 cells). Thus tissue culture miniaturizes and streamlines the selection process (Nabors and Dykes, 1985).

In Pakistan, wheat is the most important cereal and staple food of the people. It is annually grown on

8.2 million hectares with a total production of 21.5 million tones. A number of wheat varieties are grown in the country. However, data are lacking regarding the effect of salt on callogenesis and organogenesis response in wheat varieties in Pakistan. The present study was carried out to investigate the effects of sodium chloride (NaCl) salt on the callogenesis and organogenesis response of 4 Pakistani wheat varieties. The results from these *in vitro* tissue culture studies will indicate the stress tolerance potential of Pakistani varieties.

MATERIALS AND METHODS

The wheat varieties selected for this study were Arz, Pak-81, Pavon and LU-26. The study was conducted at Biotechnology laboratories, National Agricultural Research Centre, Islamabad during 1999. The explants taken were seeds of these varieties, which were exposed to different concentrations of NaCl. The seeds provide an excellent material to start with because a rigorous surface sterilization can be applied to seeds, which results in minimal contamination of the resultant callus culture. The explants were sterilized with 5% Calcium hypochlorite followed by 3 subsequent rinsing of 15 min each with autoclaved distilled water under aseptic conditions.

The medium used was Murashige (1963), (MS), modified medium (Kao and Michayluk, 1974). After preparation, the medium was poured in test tubes plugged with cotton plugs and autoclaved at 120°C and 15 lb pressure for 15 min. Salt concentrations used were 50, 100, 150 and 200 mM. The cultures were placed in environmental chamber where a photoperiod of 16 h and 25±2°C (day/night) temperature were maintained.

The callus was induced from surface sterilized wheat seeds on modified MS medium. The calli produced on callus inducing media were transferred to the regenerating media. The seed derived callus system was achieved with these steps. The four week old calli were sub-cultured using the same medium for callus proliferation. The proliferated callus was divided into small pieces (50 mg each approximately) and the pieces were transferred separately to the media with stress of NaCl. Salt tolerant response of callus lines were screened when NaCl enriched medium was used during the sub-culturing. This step was repeated 3-6 times, after four weeks time the callus was finally transferred to regenerating media for plant regeneration. The cultures were placed in environmental chamber where a photoperiod of 16 h and 25±2°C (day/night) temperature were maintained. The three kinds of culture media used in the seed derived callus culture system were modifications of MS medium. Medium I and II contains 2, 4-D (2, 4-Dichlorophenoxy acetic acid), with concentrations of 1 and 2 mg L^{-1} to induce callus, while medium III contains Naphthalene Acetic Acid (NAA) with concentrations of 2 mg L^{-1} in MS medium. The medium II and III were supplemented with NaCl stress.

RESULTS AND DISCUSSION

The factors which affected callus induction and regeneration during sub-culturing, were studied. These factors were, Variety, Incubation time and effect of Sodium chloride salt that are presented below:

Variety: Marked varietal differences for callogenesis were observed (Table 1) ranging from 25% in Arz, 34% in Pak-81, 51% in Pavon and 76% in Lu-26. The respective regeneration was minimum in Arz (5%) and maximum in Lu-26, (30%). Even without salt stress, the performance of Arz and Pak-81 was poor for both callus formation and regeneration. It indicates either the use of other medium and modification for obtaining higher frequency of callogenesis response or the unsuitability of these two varieties for stress tolerance.

Incubation time: Most varieties developed a healthy callus after four weeks of incubation. Extended incubation generally resulted in decreased efficiency of callus induction (Table 2). This may be related to hardening of callus and reduced uptake efficiency. Therefore, four weeks time of incubation was considered to be suitable for performance evaluation of calli under stress.

Effect of sodium chloride salt: When NaCl was added to the medium in concentration of 50, 100, 150 and 200 mM, percentage of calli and regeneration were decreased with increase in the concentration of salt (Table 3). At 100 mM concentration there were better results as compared to the 200 mM concentration. In case of salt, the calli grown on control medium without addition of NaCl were transferred to medium II with a stress of 150 and 200 mM concentration. Few NaCI calli turned dark brown, an indication of necrosis. However, some portions of the callus managed to grow. When this step was repeated more than 3 times, vigorously growing callus emerged from the necrotic callus. These salt tolerant callus lines continue improved growth in subsequent cultures.

Oudija *et al.* (2002) applied Salt stress to five week-old-calluses of wheat. The higher the salt stress was, the lower were relative growth and somatic embryogenesis. At 15 g salt per one liter, most calli showed necrosis and disappeared. Soft wheat tolerated more salt than durum

Table 1: Varietal differences in callus formation and regeneration from mature seeds of wheat without salt stress

Variety	Seeds inoculated	Callus formation (%)	Regeneration (%)
Arz	200	25	5
Pak-81	200	34	12
Pavon	200	51	23
Lu-26	200	76	30

Table 2: Effect of incubation time on the efficiency of callus formation

	Callus formation (%)	
Variety	4 weeks	6 weeks
Arz	25	20
Pak-81	28	23
Pavon	76	70
Lu-26	55	52

Table 3: Effect of NaCl on efficiency of callus induction and plant regeneration from mature seeds of wheat based on total seed number

Varieties	Salt concentration (mM)	Total No. of seeds	Percent of callus Formation	Percent of regeneration
Arz	50	250	23	10
	100	250	21	6
	150	250	17	4
	200	250	8	0
Pak-81	50	250	45	20
	100	250	33	17
	150	250	22	10
	200	250	30	12
Pavon	50	250	52	20
	100	250	30	13
	150	250	23	8
	200	250	10	2
Lu-26	50	250	76	37
	100	250	43	15
	150	250	21	8
	200	250	12	4

wheat. Regeneration decreased with media supplemented with 10 and 15 g L^{-1} salt concentrations. The best regeneration results were obtained in media containing 0 or 2.5 g L^{-1} of salt. Browning started to show on the seedlings at 5 g L^{-1} and was completed at 15 g L^{-1}.

Efficiency of percentage of plant regeneration was calculated on the criteria that how many regenerants were obtained from how many callus cultures. Percentage of plant regeneration declined with callus age. When NaCI was not added to the medium, efficiency of plant regeneration from callus was much higher. As a consequence, over all efficiency of plant regeneration per unit of callus initially used for subculture was lowered when salt was added to the medium.

The effects of NaCl stress on both the percentage of callus formation and regeneration are presented in Table 3. It is obvious that in every variety of wheat, with increase in salt concentration, there was a decrease in percentage of callogenesis and regeneration. Over all wheat variety LU-26 was more tolerant amongst the four varieties studied as indicated by its callus formation and plant regeneration. However, under low NaCl stress conditions (50 mM), variety Pak 81 performed better than its control (Table 1). This is in complete agreement with the results obtained by Quraishi *et al.* (2000) who have observed higher callus inductions at this concentration in Pak-81 and other wheat varieties.

Ansari *et al.* (1977) screened wheat cultivars to determine their inherent capacity of salt tolerance. It was shown that varieties of local origin (H-68, C-591) were comparatively better than their Mexican counterparts. Tomar and Punia (2003) supplemented MS medium with NaCl salt stress of 0, 0.5, 1, 1.5 and 2.0% to determine a suitable medium for *in vitro* salt tolerance screening in wheat. They observed decline in Callus growth from 0.5 to 2.0% NaCl concentration.

The effect of NaCl on callogenesis of three wheat cultivars including Pak 81 was analyzed by Quraishi *et al.* (2000). Callus induction frequency increased when 50 to 100 mM NaCl was added. Proliferation of the calli decreased with increase in salt concentration. At higher concentrations, calli lost water content and represented degenerated tissue and even died. Similar callogenesis response under NaCl stress in wheat cultivars was observed by Mahmood and Quraishi (1985) and in rice by Abbas *et al.* (1983).

Decreased proliferation leading to decay under severe salt stress can be attributed to the detrimental effects of salinity on the physiological and biochemical functions of the cells and tissues, including turgor reduction, inhibition of membrane function or enzymatic active sites, inhibition of photosynthesis, ion mechanism due to inadequate transport/selectivity mechanisms, or increased use of metabolic energy for non growth processes involved in the maintenance of tolerance (Penning de Vries, 1975; Jones and Gorham, 1983).

Zair *et al.* (2003) quantified somatic embryogenesis under salt stress conditions in wheat cultivars cultivated in Morocco. The *in vitro* selection pressure improved the salt tolerance of the cultivars as indicated by the number of somatic hybrids formed and plants regenerated.

Farrukh (2002b) studied the organic solute accumulation of wheat cultivars *in vitro* salt tolerance. The calli of two cultivars LU26S and Potohar were subjected to different salt concentrations. Callus dry weight increased with increasing salt concentrations together with the total soluble proteins and carbohydrates and free amino acids.

Qin-Jianbing *et al.* (2005) conducted studies to establish an efficient regeneration system in wheat. The induction medium containing 2, 4-D recorded higher plant regeneration frequency than that containing picloram. Calli induced on 2, 4-D showed higher frequencies of rooting, planting, green spot regenerated and number of plants regenerated per callus.

Immature embryos of wheat cultivars were evaluated for tissue culture response in three callus induction media (Haliloglu and Baenziger, 2005). Percentage of callus induction varied widely with the genotype and initiation medium used, ranging from 5.7 to 100%. Some genotypes were more embryogenic than other.

Tang *et al.* (2005) cultural embryos of mature seeds of two wheat cultivars stored at different temperatures. Callus formation and germination varied among different cultivars and germination varied among different harvest years and also among seasons in a year.

Ghannadha *et al.* (2005) studied the relationship between different traits and salt tolerance in wheat. Different concentrations of NaCl have no effect on either callus fresh weight or callus number but increasing. The salt stress decreased the callus size and increased Na^+ context of the callus. Variation of K^+ was related to tolerance or sensitivity of cultivars. Tolerant cultivars also showed higher germination percentage, coleoptile length and root length.

The results of this study are similar to the findings of other researchers which indicated that degree of callogenesis and organogenesis vary not only from variety to variety but also with concentration of the salt NaCl. There is decrease in both callogenesis and organogenesis with increase in salinity levels of sodium chloride and salt stress. These results have added valuable information regarding response of wheat varieties to salts through tissue culture. This is important in our efforts of developing salt tolerant varieties of this most important cereal of Pakistan.

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