Annals of Agricultural Science (2013) 58(2), 139–145

Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

www.elsevier.com/locate/aoas

ORIGINAL ARTICLE

Anther culture response and salt tolerance in some wheat genotypes

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Received 25 May 2013; accepted 10 June 2013
Available online 7 September 2013

KEYWORDS

Triticum aestivum L.;
Anther culture;
Multiple shoot;
Salt tolerance

Abstract Anther culture response of five bread wheat genotypes (four F_1 crosses and its parental genotype) was evaluated on four different media for their ability to initiate callus and green plantlets. Results indicated that considerable genetic variation among tested genotypes was observed. The percentage of anthers that developed calli ranged from 4.67% for the cross (Line-A × Gemmeiza-7) to 9.42% for the cross (Line-A × Misr-1) among the genotypes across the four media compared to the parental Line-A, which gave 7.67%. The cross (Line-A × Misr-2) produced the highest mean value for green plantlets (5.50%), while the cross (Line-A × Gemmiza-7) produced the lowest one of green plantlets (2.42%) compared to the parental Line-A, which gave 3.17%. Concerning NaCl concentrations, the medium without NaCl gave better response to multiple shoots as compared to the other media. The two crosses (Line-A × Misr-1 and Line-A × Gemmiza-11) with the highest response in multiple shoots had parent that exhibited very good response. The parental line (Line-A) and the cross (Line-A × Msir-1) produced the highest mean values (61.90 and 45.24, respectively) for salt tolerant index, while the control parental Line-A gave the lowest response to salt susceptibility index (0.13) as compared to its derived crosses.

Introduction

The improvement of high yielding wheat lines characterized by tolerance of salt composition covering most of the tolerant alleles and allelic combinations by plant breeding usually requires genetically pure lines. The production of these pure lines by conventional breeding practices is time consuming process (7–12 generations) and could lead to a delay in new varietals production. One area of biotechnology, anther culture technique, derived from tissue culture techniques, offers great promise for plant breeding. Anther culture (androgenesis) is to obtain haploid embryo using immature pollens (microspores) in anthers cultivated on nutrition media. This procedure usually needs short time to be conducted (only one generation) and could accelerate the production of new varieties with improved traits (Barakat et al., 2012).

Haploid plants are of great importance, which are used in achieving homozygosity in quick way, and facilitating genetic

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Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

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and breeding researches (Hassawi et al., 2005; El-Hennawy et al., 2011). The success of anther culture ability in wheat, as other crops, is found to be influenced by genotype (Andersen et al., 1987), donor plant growth conditions (Orshinsky and Sadasivaiah, 1997), the developmental stage of microspores (Haggag and El-Hennawy, 1996), pre-culture treatments, and media components (Lazaridou et al., 2005). The multiple shoot meristems used as explants, high regeneration, and transformation efficiency were reported in relatively genotypes-independent manner (Zhang et al., 1996). This shoot meristem system offers advantages to other published procedures because of its ability to obtain target tissues, vigorous regeneration characteristics of shoot meristematic cultures, and increased gene expression stability of regenerated plants (Zhang et al., 1996).

Salinity is one of the major factors responsible for low yield and restricted economic utilization of land and water resources both in arid and semi-arid regions of the world (Ghassemi et al., 1995; Arzani, 2008). The progressive salinization of soil was estimated at around 20% of irrigated land (Ghassemi et al., 1995). Approximately 20 mha of land deteriorates to zero production each year (Malcolm, 1993) mainly due to salinization. Thus, with continuous land losses and increasing population, there is tremendous pressure to avoid food shortages.

In Egypt, wheat is the most important daily food cereal; however, only 40% of its annual domestic demand can be produced (Salam, 2002). In present, cultivated land, comprises only 3% of total land area in Egypt, is already salinized. The government strategic plan is to increase the total agriculture land by adding newly reclamation land irrigated with saline underground water, due to limitation of other water resources and low precipitation (less than 25 mM annual rainfall) (Ghassemi et al., 1995). Therefore, it is necessary to disseminate newly released cultivars with more salt tolerance to be introduced for the newly reclamation land irrigated by underground water, which affected by access salt from Mediterranean and Red Seas (Shannon, 1997 and Pervaiz et al., 2002). The main aim was to study the anther culture response on different media and to assess the possibility of green plantlets to produce multiple shoots for the tested wheat genotypes in different salt (NaCl) concentrations.

Materials and methods

The present investigation was carried out at the Cell and Tissue Culture Laboratory as well as the Experimental Farm of the Agronomy Department, Fac. of Agric., Al-Azhar Univ., Nasr City, Cairo. Five genotypes of bread wheat, namely Line-A, Gemmeiza-7, Gemmeiza-11, Misr-1, and Misr-2, representing a wide range of diversity for several traits were used for this study. The pedigree of these genotypes is shown in Table 1. In 2011/12 season, the parent (Line-A) with good performance in anther culture and salinity tolerance (unpublished) was crossed with other four wheat genotypes, to obtain four crosses. The parent and its four F_1 crosses were sown at the Experimental Farm in 2012/13 season to obtain the needed anthers.

Whole tillers at boot stage were collected when most microspores were at the mid- to late-uninucleate stage of development, as assessed by acetocarmine staining of selected squashed anthers. Inter-ligule length on top of the tiller was used as an indicator of this stage. Tillers with spikes at this stage were clipped off at ground level and tagged. Then, they were put in water and maintained for 6–8 days at 4 °C in the dark. After cold pretreatment, the spikes inside flag leaves were surface sterilized with 20% chlorax solution for 7 min and rinsed 3–4 times in sterile water. Anthers were aseptically dissected out and cultures in jars containing the induction media of anther culture. Four anther culture media (Table 1), previously developed and successfully employed by other workers from wheat or novel media currently being developed for other genera, were being used in the present investigation. The anther culture medium solidified by the anther culture medium solidified by agar (6 g/L). These jars were incubated first for 5–6 weeks in darkness at 28 °C. Completely randomized design was applied in this experiment with five genotypes and 10 replicates (spikes). Each replicate contained 35 anthers from each spike, which were placed in jar.

Embryoids/callus induced from the anthers were transferred to jars containing MS regeneration medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/L NAA, 0.5 mg/L kinetin, 30 gm/L sucrose, and 6 g/L agar. These jars were incubated for 5–6 weeks at 25–27 °C with 16 h light. The number of green and albino regenerants was counted.

After regeneration, 1 cm shoot length (consisting of the shoot meristem and region of root initiation) was isolated and cultured on MS medium containing 2 mg/L BAP for 3 weeks. After 3 weeks, shoot meristems were transferred to three different culture media: modified MS medium supplemented with BAP (2 mg/L) with various concentrations of NaCl (0, 100, and 200 mM/L). The basal medium was supplemented with 30 g/L sucrose and 6 g/L agar. Three shoots were cultured vertically in each jar. Twenty-one explants were tested for each experiment with seven replications. All shoot meristems were incubated at 25–27 °C under 16 h light. The relative frequency of multiple shoot formation was calculated by dividing the total

Table 1 The pedigree of the parental genotypes of bread wheat used in this study.

No.	Name	Pedigree	Salt tolerance
1	Line-A	Line-A was obtained from Prof. Dr. M.A. El-Hennawy, Agronomy Dept., Fac. of Agric., Al-Azhar Univ.	Tolerant
2	Gemmeiza-7	CMH74A.630/SX//SERI82/AGENTCGM 4611-2GM-3GM-1GM-OGM	Sensitive
3	Gemmeiza-11	BOW"s"/KVZ"s"//7C/Seri 82/3/Giza 186/Sakha 61 GM	Sensitive
4	Misr-1	OASSIS/SKAUZ//4*BCN/3/2*PATOR CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	Sensitive
5	Misr-2	SKAUZ/BAV92 CMSS96M03611S-IM-010SY-010M-010SY-8M-0Y-0S	Sensitive