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ARTICLE

Genetic studies on tissue culture response and some agronomical traits in Egyptian bread wheat

A.G.A. Khaled [a,*], K.A. Hamam [b], M.H. Motawea [b], G.A.R. El-Sherbeny [a]

[a] *Department of Genetics, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*
[b] *Department of Agronomy, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*

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Wheat (*Triticum aestivum* L.)

Abstract The main effort of wheat breeder is the detection of genes and to merge them in a particular genotype using most suitable combination. Five Egyptian cultivars of bread wheat (*Triticum aestivum* L.) were crossed in a half diallel mating design to produce 10 crosses. The genetic potential of embryogenic callus (EC%), plant regeneration (RGP%) response and its association with heading date (HD) and grain yield per plant (GY/P) were investigated. The results showed that GY/P was significantly and positively correlated with EC% and RGP%. The combining ability analysis showed that the magnitudes of general combining ability (GCA) were higher than those of specific combining ability (SCA) for both tissue culture response and agronomic traits. The promising crosses which exhibited desirable SCA effects, showed also high useful heterosis for all studied traits. The magnitudes of additive genetic variance (σ^2A) were larger than those of non-additive ones (σ^2D) for all studied traits except for number of days to heading. The estimates of narrow sense heritability were 84.56%, 82.13%, 43.46% and 70.28% for the percentage of EC%, RGP%, HD and GY/P, respectively. The genetic similarity percents based on RAPD markers ranged from 76% to 93% between the cultivars. The UPGMA cluster analysis revealed that the cultivars could be divided into two main clusters. The range of Euclidean distances based on morphological characters among the cultivars was relatively wide (4.37–27.87), indicating relatively high amount of phenotypic variation. A significant positive correlation between Euclidean distance and RAPD distance (0.72^{**}) was found.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops in the world. It plays a remarkable role in meeting the food requirements and economic stability of the country. One of the main objectives of wheat improvement program is to generate genetically diverse germplasm that has high yield potential. The diallel cross designs are frequently

* Corresponding author. Tel.: +20 1006843428; fax: +20 932280126.
E-mail address: abdelsabour.khaled@agr.sohag.edu.eg (A.G.A. Khaled).

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used in plant breeding research to obtain information about genetic properties of parental lines or estimates of GCA, SCA and heritability in early generations and particularly suit to autogamous crops like wheat.

Tissue culture techniques are commonly used for the propagation of many plant species. It is known that in many cases, a consistent proportion of the regenerated plants differ from the original parental type when submitted to tissue-culture techniques. Conventional breeding would probably be more efficient if aided by modern tools such as somaclonal variation and molecular markers. Immature embryo of wheat has been widely used as an explant source to study embryogenesis, plant regeneration [1] and somaclonal variation [2]. Embryogenic callus trait was positively and significantly correlated with grain yield per plant in wheat [3,4], also, the earliness in wheat could be correlated with plant regeneration [5]. Quantitatively genetic parameters such as heritability and variance components are useful for designing new breeding programs and allocating resources in field performance trials. The amount of heterosis as well as the GCA and SCA effects are important considerations for hybrid breeding. Furthermore, correlation coefficients between the features are useful because they give information about the effect of the selection on other traits. Heterosis in wheat tissue cultures was reported by [6–9]. Several investigators have reported that both additive and dominance effects contribute to the variation observed among wheat genotypes [9–13]. The GCA and SCA effects were dominant and played a major role in the inheritance of days to heading and grain yield/plant [14]. [15] indicated that a superior performance of the hybrids for some traits depends on the GCA of the parents involved, that progress in improving the desired trait which will be slow if the parental selection is based on per se performance alone. For continued improvement, the selection of parents should be based on per se performance as well as combining ability and heterosis.

One of the most widely used PCR-based marker techniques is Random Amplified Polymorphic DNA (RAPD). RAPD marker is generated by PCR amplification of random genomic DNA fragments with single oligonucleotide primers of arbitrary sequence. RAPD marker analysis could assist for rapidly predicting the genetic diversity among genotypes [16,17]. The level of association between agronomic characterization and DNA marker-based genetic similarity may vary among different crop species. In corn, a close association was found [18], but in others such as, wheat, barley, oat and cotton moderate to low associations have been observed [19–21]. The present study aimed to investigate: (1) Association between tissue culture response and agronomic traits. (2) The genetic parameters of embryogenic callus induction, plant regeneration, heading date and grain yield per plant, and (3) Analysis of RAPD markers to detect the genetic variation among cultivars.

2. Materials and methods

Five Egyptian cultivars of bread wheat (*Triticum aestivum* L.), namely Gemmeiza-3, Sakha-8, Giza-168, Sakha-69 and Giza-164 were used in this study. Seeds of all cultivars were kindly supplied at the Experimental Farm of Faculty of Agriculture, Sohag University, Sohag Governorate, Egypt.

2.1. Field experiment

Half-diallel cross among the five parents was made to produce 10 crosses in the 2010/2011 winter season. In 2011/2012, seeds of the five parents and their 10 F_1 crosses were planted in a complete randomized design with three replicates. Each replication included 15 entries (5 parents and 10 F_1). Field data were recorded on number of days to heading (HD) and grain yield per plant (GY/P g).

2.2. Culture of immature embryos

Immature embryos from all genotypes (5 parents and 10 F_1) were collected 14 days after anthesis. Fifty immature embryos from each genotype in each replicate were dissected aseptically and cultured on callus induction medium with the scutellum side up. Culture induction medium contained the MS inorganic salts [22] supplemented with 150 mg/L L-aspargine, 0.5 mg/L thiamine, 1.0 mg/L 2,4-D, 20 g/L sucrose and 7.0 g/L agar. Immature embryos cultured on the callus induction medium were incubated in the dark at 27 °C for 14 days. Embryogenic calli, which were characterized as compact, yellowish and nodular, were transferred to shoot initiation medium similar to the callus induction medium, except 2,4-D concentration which was reduced to 0.2 mg/L. The cultures were incubated in the growth chamber under 12-h photoperiod at 22 °C for 2 weeks. Regenerable calli, which have green shoot primordia covering the surface, were transferred to hormone-free MS medium and incubated under the same conditions in the growth chamber. The good developed plantlets were transferred to the greenhouse for further growth.

Data were recorded on the percentage of embryogenic calli (number of embryos forming callus per number of immature embryos cultured on the medium × 100) and percentage of regenerated green plants (number of regenerable calli that produced whole plants with a well developed root system per transferred differentiating calli × 100).

2.3. RAPD marker technique

2.3.1. Genomic DNA extraction and PCR procedures

Fresh leaves were frozen in liquid nitrogen, lyophilized, and ground to a fine powder using mortar and pestle. DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method according to [23]. RAPD technique was conducted using 9 arbitrary 10-mer primers (Metabion International AG, Germany).

The RAPD assay was performed in a 25 µl volume containing 12.5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 3.5 µl of primer 5 pmol, 7 µl of free nuclease water and 2 µl of 150 ng DNA template. The Thermal Cycler (Primus 25, Germany) was programed by an initial denaturation cycle at 94 °C for 5 min. The following 45 cycles were composed of: denaturation step at 94 °C for 1 min, annealing step for 1 min 45 s at 38 °C and elongation step at 72 °C for 2 min. The final cycle of polymerization was performed at 72 °C for 7 min. The amplification products were electrophoresed in a 1% agarose gel stained with 0.4 µl ethidium bromide. The amplified fragments were visualized and photographed using UVP Bio Doc-It imaging system (USA).