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EFEECT OF AGE OF EMBRYOGENIC CALLUS ON PLANT REGENERATION IN LOCAL CULTIVARS OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

High frequency regeneration system is a prerequisite for production of transgenic plants. The present study was conducted to study the age of callus on high frequency regeneration protocol for seven different wheat (*Triticum aestivum* L.) cultivars viz. Chakwal-97, Inqualab-91, Punjnand-2000, Manthar-2002, Kohsar-95, Margalla-99 and C-591. Mature embryos of these cultivars were inoculated on MS (Murashige and Skoog, 1962) and N_6 (Chu, 1978) media supplemented with 2.5 mg/l 2, 4-D. Callus induction started after two weeks. These calli were placed on regeneration medium supplemented with BAP at 5 mg/l and IAA at 1mg/l) after 14, 18, 22, 26, 30, 34, 38, 42, 44, 48, 52, 56 and 60 days. Regeneration frequency was noted after 6-8 weeks of inoculation on regeneration medium. It was found that regeneration frequency was highest i.e., 95% by Chakwal-97 in 26 days old calli, whereas lowest at 48 days i.e., 13%. From these studies it was concluded that 3-4 weeks old mature embryo derived calli were more effective for highest regeneration frequency.

Introduction

Recent progress in genetic manipulation of plant cell has opened new possibilities in crop improvement. A more recent and stable way is through tissue culture in which crop species can be improved without interfering with a large portion of genome and without introducing alien or exogenous genetic material (Lacock & Botha, 2000). The plant tissue culture provides innovative technique for the development of crop varieties having desirable traits. These traits take less time, less expensive and require less space than conventional breading methods. New varieties of cereals, particularly wheat and rice, can evolve in exactly half the time required by conventional breeding techniques. Winding the genetic pool of a crop species is a genuine need of the breeder, and tissue culture certainly a powerful tool, which can be hammered in this regard. Tissue culture can therefore, has a very strong impact on agriculture (Quraishi *et al*., 2000).

The application of many biotechnological methods in cereal improvement involves the establishment of standardized protocol for the production of embryogenic callus, which can be used for all the biotechnological techniques (Vasil & Vasil, 1999). By using these methods, large numbers of new varieties of wheat were introduced which have higher production, disease resistance and are stable for adverse climatic conditions. These achievements play an important role to fulfill food requirements. Wheat cell and tissue culture research depends upon reliable callus culture and plant regeneration procedures. Plant regeneration is one of the more critical steps of plant transformation (Keresa *et al*., 2001). Plant regeneration frequency depends upon specific combination of media used and genotype of wheat. Similarly, age of callus also play critical role in this regard (Saad *et al*., 2004).

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The objective of the present study was to investigate the effect of age of callus on plant regeneration in seven promising cultivars *viz*., Chakwal-97, Inqualab-91, Punjnand-2000, Manthar-2002, Kohsar-95, Margalla-99 and C-591 of wheat by exploiting more effective combination of various growth regulators and addition of osmoticum. The high regeneration frequency, which was the main aim of the study, would lead us to genetic improvement of these cultivars.

Materials and Methods

The present study was conducted in Agricultural Biotechnology Programme (ABP), NARC, Islamabad. Mature healthy seeds of seven cultivars viz., Chakwal-97, Inqualab-91, Punjnand-2000, Manthar-2002, Kohsar-95, Margalla-99 and C-591 of wheat (*Triticum aestivum* L., 2n=6x=42) were washed with commercial detergent (Zip) under tap water. The seeds were then surface sterilized with a brief (30 seconds) rinse with 95% ethanol followed by 20 min., vigorous wash with 30% Clorox® (1.5% sodium hypochlorite solution), plus a drop of Tween 80. The seeds were thoroughly washed six times with sterile distilled water in a laminar air-flow cabinet and were transferred to sterile Petri dishes having filter paper for drying. Murashige and Skoog (1962) and Chu (1978),salts and vitamins, 3%(w/v) sucrose and 0.12% (w/v) Gelrite® were used as callus induction medium supplemented with different concentrations (2.5 mg/l) of 2,4-dichlorophenoxy acetic acid (2,4-D). The media were adjusted to pH 5.75 and autoclaved for 15 min., at 121°C.

Sterilized seeds of all varieties were inoculated on the gel solidified, autoclaved MS and N6 media supplemented with 2,4-D, in glass tubes (18 mm in diameter and 150 mm in depth). 8 ± 1 ml medium was taken in each culture vessel and one seed was planted per culture vessel under sterilized conditions. Cultures were transferred and maintained in environmentally controlled room under continuous illumination of 1500 lux emitted by general electric fluorescent tubes. Temperature was maintained at 25 ± 3°C throughout the growth period for optimum growth and to control contamination. About 4-5 weeks were permitted for adequate induction and growth of the calli for all genotypes. At the end of each culture passage, non-embryogenic calli, recognized on the basis of visual estimates by naked eye, was dissected away from the embryogenic callus and discarded.

The plant regeneration ability of all genotypes was assessed using MS salts and vitamins, 3% (w/v) sucrose and 0.2% (w/v) Gelrite®. Different combinations of growth regulators, that is, BAP (6-benzylaminopurine) at the rate of 5 mg/l and IAA (Indole 3-acetic acid) at 1 mg/l were assayed to induce shoot and root differentiation and subsequent regeneration of plants from different aged calli.

Calli were carefully cleared of any surrounding non-embryogenic portions after 14, 18, 22, 26, 30, 34, 38, 42, 44, 48, 52, 56 and 60 days, divided into small pieces (4-5 mm in diameter) and placed on above mentioned combinations of BAP and IAA. The calli were inoculated on the regeneration medium in glass tubes and also in glass jars, separately, where each treatment comprised 6-7 cultures. Calli bearing green spots and the total number of regenerates were counted after a time interval of 6-8 weeks. Each developing green shoot with an initiated root system was counted as one plant.

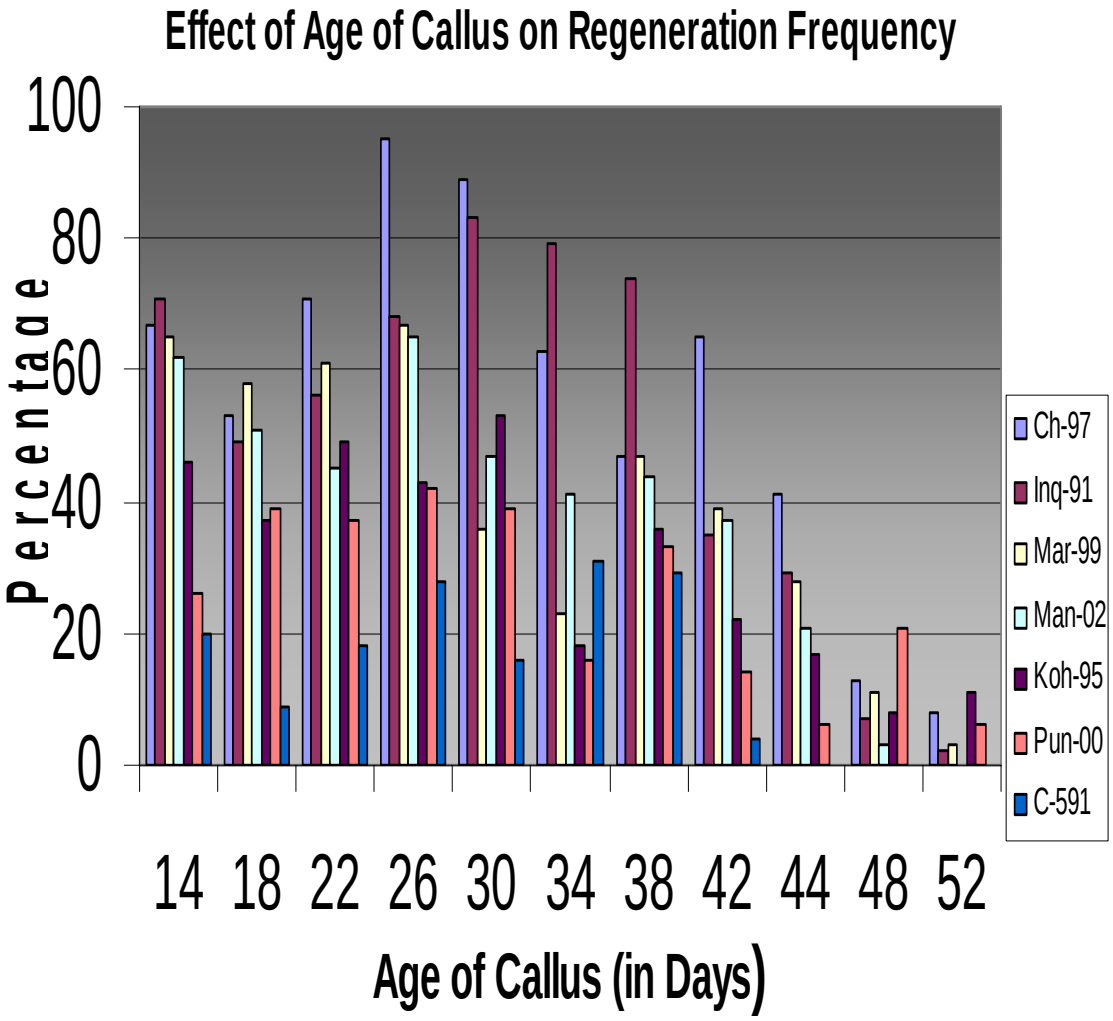

Fig. 1. Effect of age of callus on Regeneration frequency on MS media supplemented with 1 mg/l IAA and 5 mg/l BAP.

Results and Discussion

Age of the callus is very important factor for its regeneration. Generally calli with less age have more totipotency as compared to old calli (Rashid *et al*., 1994). In present study, it was found that the best age for regeneration is between 22 to 30 days old calli (Fig. 1). Callus of age less then 22 days was either too small or fragile that they can not survive, so their regeneration frequency is lesser (Fig. 3). On the other hand, callus with age more than 30 days has lost their regeneration ability due to repeated cell divisions. But this optimum age was hormone and media specific. BAP is an important cytokinins in conferring competence to regenerate in cereals, plays an efficient role in shoot differentiation at low level along with IAA (Ignacimuthu, 1997, Raja *et al.*,2008). Shoot forming cultures have been able to produce viable plants by subsequent rooting (Bhaskaran & Smith, 1990).

Kohsar-95 gives the best results at the age of 24 days old calli. These results showed less regeneration frequency then that of the results with BAP and IAA but these results were in contrast to that of Shrivasta and Chawla, 2001, which might be due to callus age, genotype and change of chemical nature of hormone. Plant regeneration frequency changes by changing chemical nature of growth regulator, in this regard natural hormones were found to be more efficient than the synthetic one (Elwafa *et al*., 1999)

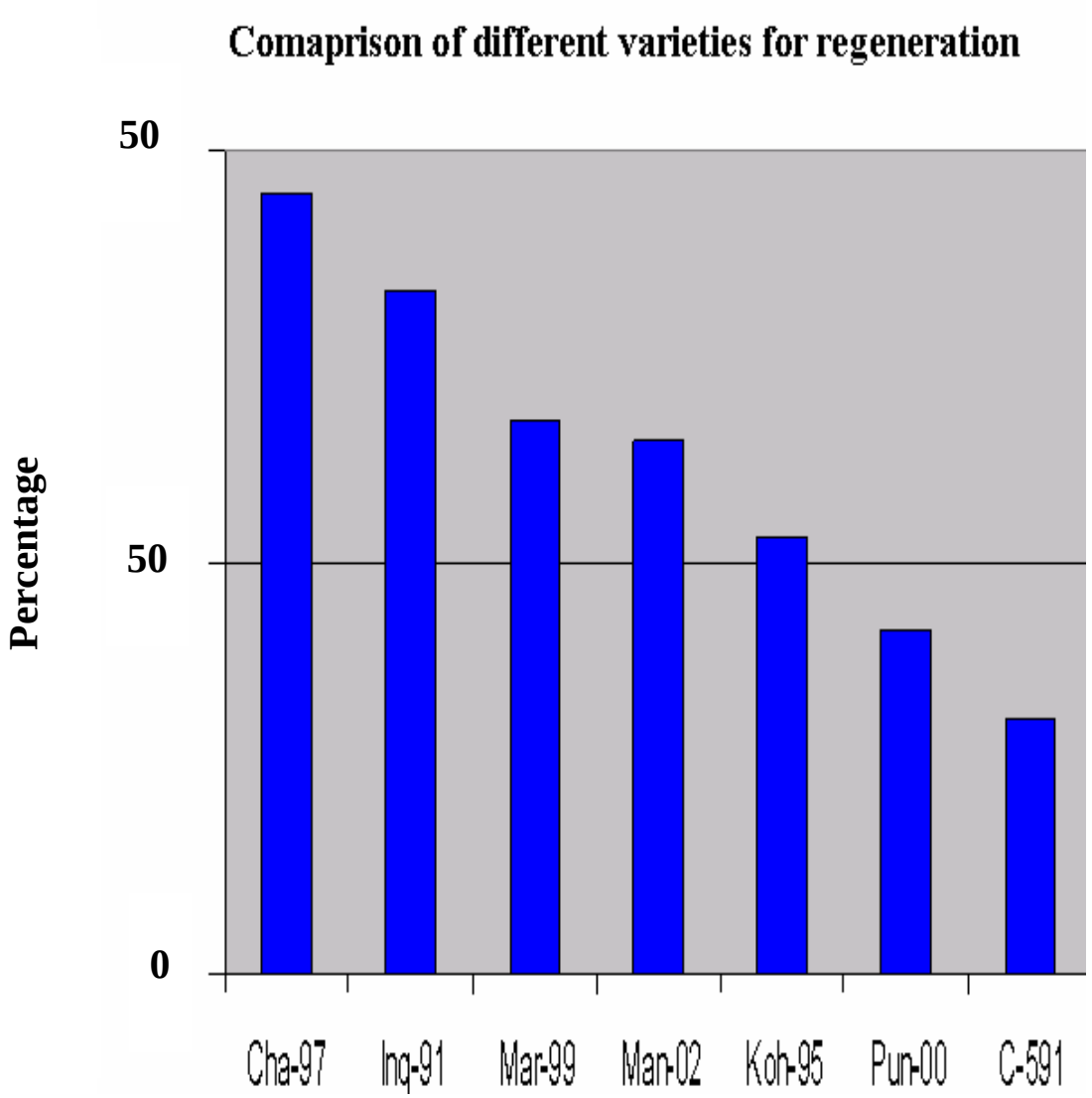

Fig. 2. Comparison of different varieties on MS medium supplemented with 1 mg/l IAA and 5 mg/l BAP for regeneration efficiency.

Regeneration frequency (Fig. 2) is also dependent on genotype of same specie; it may be due to the fact that plantlet regeneration might be controlled by the genetic system (Kamil *et al*., 2005). Different wheat varieties showed different behavior i.e., Chakwal-97 (Fig. 4) showed highest regeneration frequency (94%) at the optimum age of 26 days, second most was found to be Inqualab-91 (84%) at the optimum age of 30 days. Similarly, least regeneration frequency was shown by the C-591. Similar results were also observed with some other varieties by Maralappanavar *et al*., 2000. Conditions optimum for plant regeneration in one variety fails to develop plants in another variety of same species (Bhaskaran & Smith, 1990). Different varieties had different genotypes, which respond differently in changing environmental conditions. Similarly, some varieties loose their totipotency slowly with age as compare to the other ones (Saad *et al*., 2004). This was related to variations in endogenous hormones levels. The application of exogenous growth regulators, in combination with those of endogenous origin produced under the specific internal genetic control and environmental influence can alter the hormone balance in favour of organogenesis or embryogenesis in lieu in their normal mode of development, eliciting a specific response (Bhaskaran & Smith, 1990).

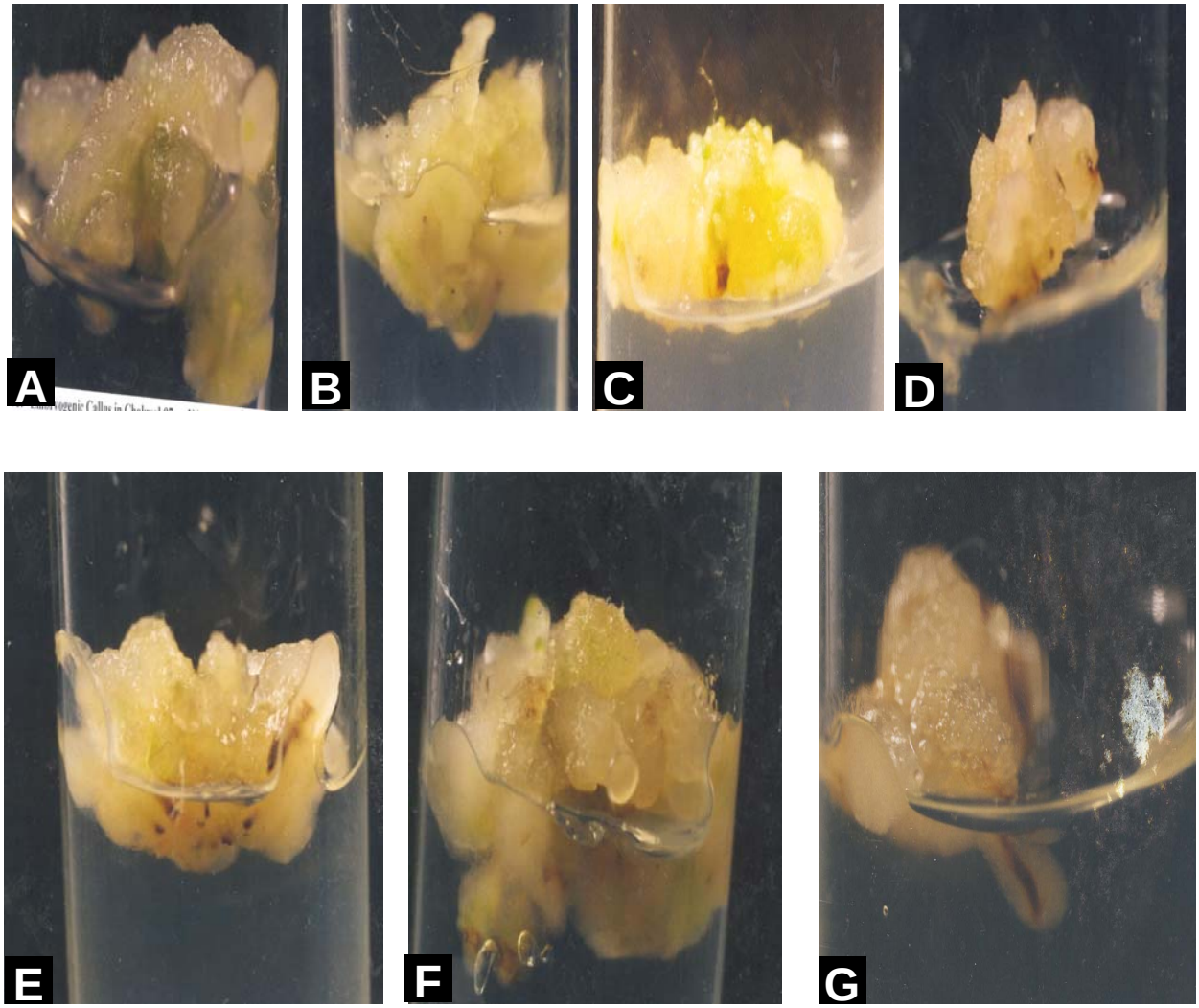

Fig. 3. 24 Days old calli of; (A) Chakwal-97, (B) Inqualab-91, (C) Marghalla-99, (D) Manthar-2002, (E) Kohsar-95, (F) Punjnand-2000, (G) C-591.

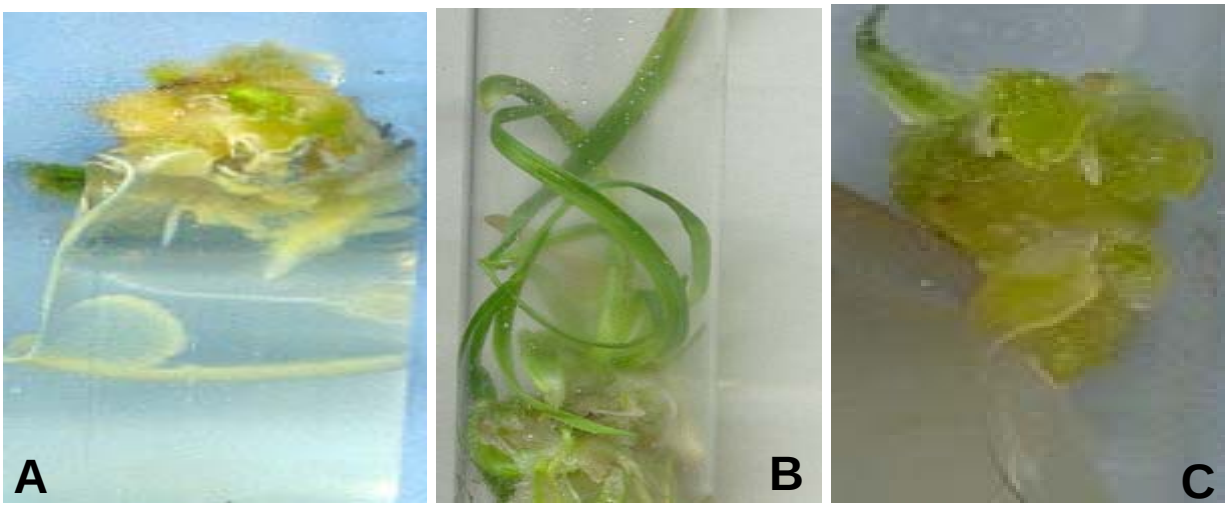

Fig. 4. Different steps of regeneration of Chakwal-97 on MS medium; (A) Green spotting, (B) Shoot induction, (C) Plant regeneration.

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