Effect of 2, 4-dichlorophenoxyacetic acid on callus induction in wheat (*Triticum aestivum* L.) genotypes

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Abstract

Wheat is a member of family poaceae. Mature healthy seeds of four genotypes of wheat viz., PBW343, PBW373, RAJ 3765 and HD 2687 were inoculated on the MS media supplemented with 2, 4-D (2, 4-Dichlorophenoxy acetic acid). Four different combinations of 2, 4-D ranging from 5-8 mg L^{-1} was used for callus induction in four Indian varieties. Higher levels of 2,4-D were found to inhibit callus proliferation. Maximum callus induction was obtained in PBW343 (90.0%) at 6.0 mg L^{-1} 2, 4-D followed by HD2687 (65.0%). Good embryoid production was apparent in third and fourth weeks of callus subculture. Average fresh weights of the calli for PBW 343 and HD2967 were 0.31 and 0.27 g per explant source, respectively.

Keywords: Mature seeds, wheat, callus, 2,4-D

Introduction

As an abundant source of energy and proteins, wheat is one of the most important food crops for the world population. However, the research on development of transgenic in wheat is still under primitive stage due to its own lacuna. To date, a significantly high throughput wheat transformation system via *Agrobacterium* is lacking in India both in the public and private sector (Chauhan e*t al.*, 2011). For production of transgenic plants, an efficient *in vitro* regeneration system is prerequisite. Highly efficient and reproducible regeneration of plants from callus and suspension culture was established in 1980's largely through the efforts of Vasil and coworkers in Florida. Genetic transformation enables the introduction of novel genes directly into locally adapted cultivars to create new genetically modified varieties (Jones *et al.*, 2005), using tissue culture protocols as their base line. These procedures require that a whole plant should be regenerated from isolated cells or tissues. Many protocols have been developed but in wheat they are very much genotypic dependent (Bhalla *et al.*, 2006). Due to these complexities, there is need to standardize optimum regeneration protocol for each activity. *In vitro* regeneration of wheat is possible from different explants such as mature and immature embryos, seeds, endosperm, leaves, leaf bases and root tips (Sarker and Biswas, 2002). Among these, immature embryos and scutella are considered as best sources for regenerating whole plants not only in bread wheat (Zale *et al.*, 2004), but also in durum wheat (He and Lazzeri, 2001). Mature embryos and seedling explants, which are readily available at all times, are the least frequently used explants because of low frequency of callus induction. Mature embryos can either be dissected (Yu *et al.*, 2008) or used directly (Özgen *et al.*, 1998). Callus induction and regeneration from immature and mature embryos and immature inflorescences have proved to be genotype-dependent and strongly influenced by the components of the media used (Fennell *et al.*, 1996). As a member of the *Graminae* family, wheat is a recalcitrant crop that limits the utilization of tissue culture techniques for crop improvement. The specificities of tissue culture of wheat greatly limit the use of chloroplast transformation technology in wheat. If an efficient and reliable protocol for regeneration from mature embryogenic culture is available, it would facilitate the introduction of agronomically important traits of wheat and research could be carried out on wheat transformation throughout the year. Keeping this in view, the present study was undertaken with the objective to optimize the cultural conditions and to find suitable 2,4-D levels for efficient callus induction and growth in four (PBW343, PBW373, RAJ 3765 and HD 2687) Indian wheats varieties.

Material and methods

Mature wheat embryos of four wheat genotypes; namely HD 2687, RAJ 3765, PBW 343 and PBW 373 were used as explants for callus induction. For sterilization of plant material, mature seeds were washed properly with Tween-80 or Teepol and soaked in distilled water for 2 hours before embryo dissection, so that embryos can swell and become distinctly visible. The soaked seeds were surface sterilized with 0.1 per cent mercuric chloride solution for 7-8 minutes followed by 5-6 times thorough washings with sterilized distilled water for removing the sterilant. Mature embryos were asepetically excised from the caryopsis and placed on callus induction medium in sterile petridish keeping the scutellum side up. Twenty mature embryos were placed in petridish of each genotype and were sealed with parafilm. The callus induction media contained agar solidified MS (Murashige and Skoog, 1962) media supplemented with 4 different doses of 2, 4-D (5, 6, 7, 8 mg l^{-1}), when callus induction began the petridish

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were transferred to light with 16 hours photoperiod under the same temperature regime.

The culture was checked daily to note the initiation and development of calli.

Callus induction (%) = (The number of callus induced/ Total number of explants inoculated)*100

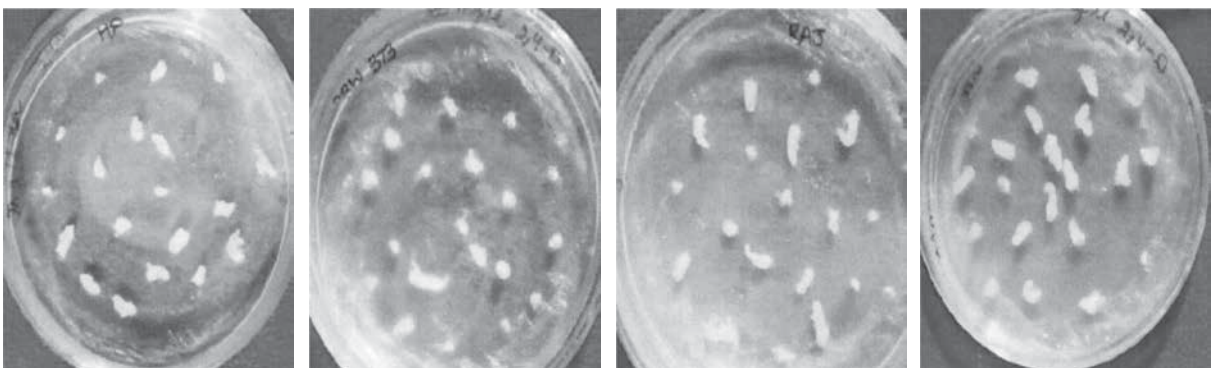
Results and discussion

Mature embryos of four genotypes were cultured on MS medium containing different concentrations of 2, 4-D (5-8 mg L^{-1}) under dark conditions to identify the most responsive genotype and the callus induction medium. Callus initiated from scutellum of mature embryo after 4-5 days and lasted up to four weeks rapidly, following less frequent proliferation during subsequent time period which affect both callus quantity and quality. Callus was friable and creamish white in colour as shown in figure 1. Some genotypes had low callus induction (Raj 3765) frequency as it varied from 25.0 per cent to 90.0 per cent depending on the cultivar in MS+2, 4-D medium concentration. Medium supplemented with 6.0 mg L^{-1} of 2, 4-D produced maximum calli (Table 1). On further increasing the concentration of 2, 4-D, callus induction decreased. Among the genotypes, maximum callus induction and callus growth was found in PBW343 (90.0 %) and was minimum in RAJ 3765 (55.0%). Sarkar and Biswas (2002) reported that mature embryos of wheat showed best response for callus induction in MS medium supplemented with 6.0 mg L^{-1} of 2, 4-D. For callus induction in mature embryos of winter wheat, Ozgen *et al.* (1998) used MS medium supplemented with 8 mg L^{-1} 2, 4-D. Fazeli-nasab *et al.* (2012) has used MS medium supplemented with 10 mg L^{-1} 2,4-D and 30 g L^{-1} sucrose and different levels of ABA (0-8 mg L^{-1}). In our study, 8 mg L^{-1}, 2, 4-D medium was found to be least effective for callus induction for all the four genotypes used. These variations of callus induction at different concentrations of 2, 4-D may be due to different genotypes of wheat varieties as each variety show a different response to tissue culture techniques. Yasmin *et al.* (2009) found that the mature embryos failed to initiate any type of calli at low concentrations of 2, 4-D resulting only in initial swelling. Tomar and Punia (2003) reported that increase of 2, 4-D concentration in culture media produced good callus from mature embryo of wheat

Table1. Callus induction from mature embryos of four cultivars of wheat

Genotype	PBW343				HD2687				RAJ 3765				PBW 373			
MS + 2,4 D (mg/l)	5	6	7	8	5	6	7	8	5	6	7	8	5	6	7	8
% callus induction	55	90	80	45	35	65	55	25	35	55	45	25	35	60	50	20
Average weight of callus per mature embryo (g)	0.0098	0.3162	0.1871	0.1009	0.0087	0.2711	0.192	0.0876	0.0041	0.1091	0.0912	0.0231	0.0065	0.1623	0.115	0.0871

In conclusion, our studies indicate that cultivars varied significantly in their response to callus induction. Optimum concentration of 2, 4-D should be used during callus induction as higher concentration inhibit callus proliferation while lower concentrations allowed morphogenesis to occur. Though, good callus induction limited the regeneration capacity. Optimizing cultural conditions, development of efficient protocols for *in*



a b c d

Fig 1. Callus induction from the mature embryos of four cultivars (a) HD 2967 (b) PBW373 (c) RAJ 3765 (d) PBW 343.

vitro cultures and screening of wheat genotypes for tissue culture response may play an important role in crop improvement by shortening the time needed to release new varieties or introducing new variability in the existing material. Impact of such technologies is tremendous and hence demands its integration with conventional plant breeding methods.

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