Cultivar specific response of callus induction and plant regeneration from mature embryos in different elite Indian wheat

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Abstract

To develop a successful cultivar-independent in vitro plant regeneration protocol in wheat, mature embryos of nine Indian elite wheat (Triticum aestivum) cultivars were taken to establish a reliable effective, reproducible callus culture and plant regeneration procedure. Two different auxins, naphtalenacetic acid (NAA) and dichlorophenoxyacetic acid (2,4-D) and two different cytokinins, 6-Benzylaminopurine (BAP) and kinetin (Kn) were assessed in several combinations for their effect on callus induction and plant regeneration from mature embryos in nine elite Indian wheat cultivars. The cultivar-dependent substantial variances were noted in per cent of regeneration response. The callus induction was assessed in terms of size of callus induced in seven days of initiation. Induction of callus did happen in all cultivars on all media combinations tried, though there was difference in size of callus induced in different cultivars cultured with different combination of growth regulators.

However, the induced callus, irrespective of cultivar when nurtured on MS media supplemented with 2,4-D (4.0 mg/L) and NAA (1.0 mg/L or 2.0 mg/L) showed prominent callus growth. There were significant differences among the nine cultivars in regeneration of shoots from embryonal callus on the various shooting media used. In spite of cultivar dependent response, the MS media supplemented with kn (2.0 mg/L), NAA (0.5 mg/L) and BAP (0.5 mg/L) was identified as the best media for shoot regeneration for some of the elite Indian cultivars.

Keywords: Bread wheat, *in vitro* culture, mature embryos, callus, regeneration.

Introduction

Wheat (*Triticum sp.*) ranks as the second most widely consumed food crop in India and *T. aestivum* (bread wheat) is the most common wheat species cultivated commercially. It is an important source of energy (1368 kJ), contains proteins (12.6%), carbohydrates (71.2%), vitamin B, fibre etc. To feed the growing Indian population, a target production of 105 million tonnes in wheat is to be met by 2025.

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Unfortunately, cultivation of wheat is greatly affected by a wide range of biotic and abiotic stresses. It would be difficult to meet this target with conventional plant breeding strategies alone. Conventional practices coupled with genetic engineering and plant transformation can lead to a significant increase in wheat production by improving their characteristics and resistance/tolerance to various biotic and abiotic stresses. Most methods of plant transformation applied to development of genetically modified crops require that a whole plant is regenerated from isolated plant cells or tissues that have been genetically transformed.[26] Tissue culture technology helps to aseptically generate numerous regenerable cells and plants *in vitro* which are easily accessible to transformation studies.

The success of cell and tissue culture research depends upon a reliable callus culture and plant regeneration procedures. The frequencies of callus induction and plant regeneration in tissue culture of wheat are highly influenced by the cultivar,[6,9,30,32] culture medium[9,17,19] and source of explant such as mature embryos,[13,15,23] mesocotyls,[34] immature embryos,[1,5,8,30] apical meristems,[20] seeds,[10] immature inflorescences[18,24,28,31] and immature leaves.[2,36]

The highest frequencies of callus and plant regeneration have been obtained from the culture of immature embryos in wheat. Immature embryos are, therefore, known to be the best explants for efficient regeneration from callus culture of wheat. Immature embryos have been used frequently as an explant source in wheat tissue culture, but ready availability of immature embryo throughout the year is a major problem. Moreover, the specific suitable stage of immature embryo also becomes a limiting factor. Mature embryos which are readily available at all times are the least used explant sources because of their low frequency of callus induction. However, some new techniques such as the endosperm supported callus induction method have been successfully used in callus induction from mature embryo cultures.[23] Callus induction and regeneration in wheat have been reported to be cultivar specific. Indian cultivars have been highly recalcitrant in comparison to their other counterparts.[4]

In the present study we aimed at improving the callus development and regeneration response in nine elite cultivars of Indian bread wheat for further use in development of transgenic wheat.

Material and Methods

Seed material: Seeds of nine elite Indian cultivars namely cv. DBW 14, DBW 17, DBW 38, DBW 39, HD 2967, HD

2894, HD 2733, HD 2932 and HD 2987 were obtained from Division of Genetics, IARI (Indian Agricultural Research Institute), New Delhi, India.

Preparation of explants: The seeds of above mentioned nine cultivars were taken to improve and develop regeneration. Seeds of each cultivar were thoroughly washed in Tween-20 solution with continuous shaking for 20 minutes followed by washing with sterile distilled water. To remove seed borne fungal contamination, seeds were kept in circulating water bath at 45°C for 45 minutes. The seeds were surface sterilized in aseptic condition with 70% ethanol for 3 minutes followed by 4% sodium hypochlorite (NaOCl) solution for 20 minutes. Traces of NaOCl were washed off by repeated rinsing with sterile distilled water. Mature embryos were then excised aseptically from seeds and then cultured on plant culture media.

Preparation of media for callus induction and development: Callus induction and development, the effects of ten induction media were compared. The MS media was taken as basal medium and supplemented with 30% sucrose and other essential nutrients[21]. Agar @ 8 g/L was used as a solidifying agent, the pH was adjusted to 5.8 and autoclaved at 121°C and 1.1 kg/cm^2 pressure for 20 minutes. The growth regulators (naphthaleneacetic acid: NAA; 6-Benzylaminopurine: BAP; 2,4-dichlorophenoxyacetic acid: 2,4-D; Kinetin: Kn) were filter sterilized and added in the sterile media individually or in several combinations (table 1). The excised mature embryos were placed with the scutellum upwards and cultured for 3-4 weeks at 25±1°C temperature in dark conditions for the callus induction and development.

Media for regeneration of shoots: Shoot regeneration media was prepared as for callus induction and development with difference in the growth regulators. The growth regulators (NAA, BAP, Kn) were filter sterilized and added to the sterile media individually or in several combinations (table 2). Calli were transferred to the shoot regeneration media and cultured at 25±1°C in a 16 h light/8 h darkness for 3-4 weeks. The experiments were planned with completely randomized design and three replicates per cultivar were used for analysis of the effect of cultivar and medium on culture responses by determining variance and least significant-difference.

Results

The mature embryos of all nine wheat cultivars were cultured *in vitro* and evaluated for callus induction and growth and shoot regeneration. The media vs. cultivar response to *in vitro* culture in wheat was evaluated by a sequential two step procedure.

In the first step, MS basal media supplemented with ten different combinations and concentration of several growth regulators were tested for callus induction and growth development followed by a second step wherein five different combinations of growth regulators were tested for shoot regeneration using the callus induced in the first step.

Callus induction and development: To identify the media best suited for inducing callus and its development, embryos were cultured in media supplemented with four growth regulators, NAA, 2,4-D, BAP and Kn in different combinations and concentration. The percentage of callus induction was recorded after seven days. Cultivar dependent response was observed where callus induction frequencies varied from 30.0 % to 98.0 % in the nine cultivars tested. The data was analyzed statistically and the media on which callus induction was more than 85.0 % were considered as good media for callus induction. Media containing BAP @ 1.5 mg/L and termed as $B_{1.5}$ media was observed to be good callus induction media for cv. DBW 14, DBW 38, HD 2967 and HD 2733 as there was 91.3-98.0% callus induced in these cultivars on media $B_{1.5}$. Similarly, media supplemented with BAP@ 2 mg/L (media termed B_2) were observed to be a good media for DBW 38, HD 2967, HD 2733 and HD 2987 with callus induction in the range of 91.7-97.7%.

In contrast, the media supplemented with NAA@ 4 mg/L (media termed N_4) were recorded as an abortive media for callus induction for all tested cultivars and callus induction of embryo cultured on this media ranged from 31.0% to 80.7%. A few cultivars e.g. DBW 38, HD 2967, HD 2733 and HD 2932 had great response to more than one medium for callus induction (table 3). When the cultured embryos were allowed for longer incubation up to 10 days in the same media, there were 85% or more callus inductions in most of the cultivars (table 4). Thus, media and cultivar influenced only early callus induction from mature embryos in wheat.

Development of callus was also significantly influenced by combinations of growth regulators and was observed to be cultivar dependent. The percentage of overall callus growth varied from 13.0% to 97.3% (table 5). Although the embryos cultured in media $B_{1.5}$ and B_2 showed good response in callus induction, further growth and development were retarded on these media.

However, media supplemented with 2,4-D @ 4 mg/L and NAA @ 1 mg/L (media termed as D_4N_1) as well as media supplemented with 2,4-D @ 4 mg/L and NAA @ 2 mg/L (media termed as D_4N_2) were identified as best media for further callus development and growth of embryonal callus induced irrespective of cultivar (fig. 1). Higher percent of embryonal callus growth was observed for all the cultivars on D_4N_1 and D_4N_2 as compared to other media tested. A few cultivars (DBW 38, HD 2733, HD 2932 and HD 2987) also showed good response in callus development and growth in media supplemented with 2,4-D @ 8 mg/L (media termed as D_8) and media supplemented with 2,4-D @ 5 mg/L and kinetin @1 mg/L (media termed as D_5K_1) media (table 5).

In contrast, media supplemented with 2,4-D @ 4mg/L and BAP @1 mg/L (media termed as D_4B_1) ; media

supplemented with 2,4-D @ 4mg/L, BAP @1 mg/L and kinetin 1 mg/L (media termed as $D_4B_1K_1$) and media supplemented with 2,4-D @ 4 mg/L, NAA@1 mg/L and BAP 1 mg/L (media termed as $D_4N_1B_1$) were observed to be unsuitable for further callus development and growth for all the cultivars tested (fig. 2).

Additionally, media dependent growth variation of callus with respect to size was observed in all cultivars. The average callus size for cultivars tested was varied from 0.3 mm to 7.7 mm after 3 weeks of incubation. Embryonic callus did not show good response in callus growth when cultured on D_4B_1, $D_4B_1K_1$ and $D_4N_1B_1$, whereas all other media supported good embryonic callus growth. Media, D_4N_1 and D_4N_2 were found to be cultivar independent media for callus growth (table 6). In brief, D_4N_1 was found to be the best callus growing media for cv. DBW 17, DBW 38, DBW 39, HD 2967, HD 2733 and HD 2987 whereas D_4N_2 was best for cv. DBW 14 and HD 2894. But exceptionally, the best callus growth of cv. HD 2932 was found in N_4 media (table 6).

Shoot regeneration: The well-developed calli of different cultivars were transferred into different shoot generation media (table 7). Cultivar dependent shoot regeneration response was distinctly observed in wheat embryonic callus while media compositions also seemed to influence shoot regeneration. Most of the cultivars (except HD 2967) responded in B_2 media and regenerated shoots from 12.8% – 80.9% callus (table 7). There was distinct variation in the extend of shoots regenerated from embryonic callus among the different wheat cultivars tested.

Cultivar HD 2987 had maximum response in B_2 media (fig. 3) whereas lowest regeneration was found in cultivar DBW 14. Except cultivars DBW 17 and HD 2894, the embryonic callus of all the cultivars regenerated shoots in more than one medium whereas both, DBW 14 and HD 2894 responded only in B_2 media. The extend of response on this media was very poor as only few weak shoots were regenerated which too did not grow further. Additionally, the three cultivars HD 2967, HD 2733 and HD 2932 which had shown distinct media based varied response for callus induction, responded well with respect to shoot regeneration in all the shoot regeneration media tested.

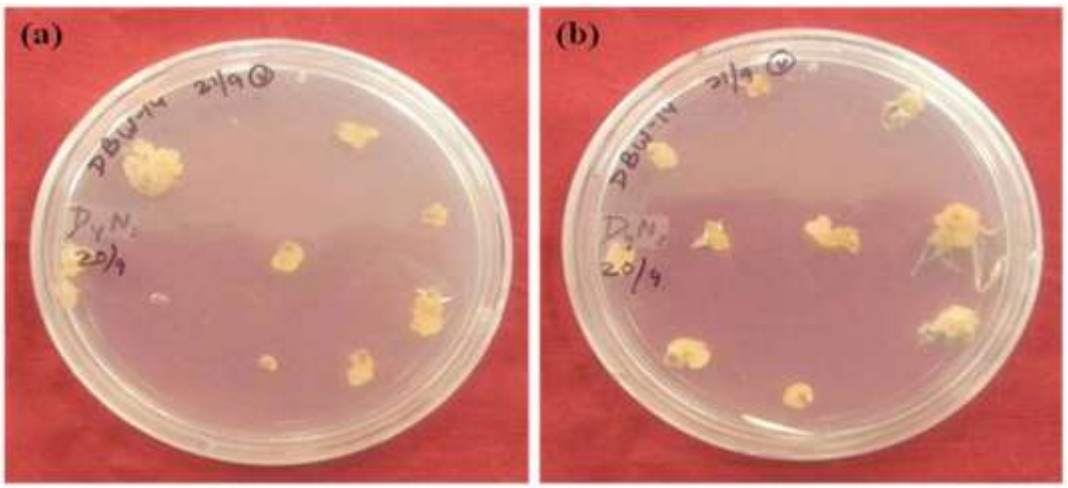

Fig. 1: Embryonal callus growth in cv DBW14 on different media (a) MS + 2,4-D @ 4 mg/L + NAA@ 1 mg/L (b) + 2,4-D @ 4 mg/L +NAA @ 2 mg/L

Table 1
The list of media used for callus induction and development

Media Code	Growth regulators combination (per L)	References
$B_{1.5}$	BAP:1.5 mg	Present study
B_2	BAP: 2 mg	
D_4B_1	2,4-D: 4 mg, BAP: 1 mg	
$D_4B_1K_1$	2,4-D: 4 mg, BAP: 1 mg, Kinetin: 1 mg	
$D_4N_1B_1$	2,4-D: 4 mg, NAA: 1 mg, BAP: 1 mg	JadhavPravin et al[14]
D_5K_1	2,4-D: 5mg, Kinetin: 1 mg	Coskun et al[7]
N_4	NAA: 4 mg	Nasircilar et al[22]
D_8	2,4-D: 8 mg	Turhan and Baser[33]
D_4N_1	2,4-D: 4 mg, NAA: 1 mg	
D_4N_2	2,4-D: 4 mg, NAA: 2 mg	

Table 2
The list of media used for shoot regeneration

Media Code	Growth regulators combination (per L)	References
B_2	BAP: 2 mg	Present study
$B_2K_{0.5}$	BAP: 2 mg, Kinetin: 0.5 mg	
B_2K_1	BAP: 2 mg, Kinetin: 1 mg	
$B_{0.5}K_2N_{0.5}$	BAP: 0.5 mg, Kinetin: 2 mg, NAA + 0.5 mg	Jadhav et al[14]
$B_{0.225}$ +36 M	BAP: 0.225 mg, Maltose: 36 g	Hensel et al[12]

Table 3
Percent of callus induction in different wheat cultivars after seven days of incubation

Media code	DBW-14	DBW-17	DBW- 38	DBW-39	HD-2894	HD-2967	HD-2733	HD-2932	HD-2987
$B_{1.5}$	96.3^{a}	73.0^{bc}	92.0^{a}	80.0^{b}	43.0^{cd}	91.3^{a}	98.0^{a}	61.0^{de}	70.3^{b}
B_2	79.7^{b}	60.7^{d}	92.0^{a}	80.7^{b}	70.0^{b}	91.7^{a}	97.7^{a}	71.7^{cd}	97.3^{a}
D_4B_1	61.0^{bcd}	65.0^{cd}	91.7^{a}	90.3^{a}	30.0^{e}	70.3^{bc}	69.7^{cd}	80.0^{bc}	42.3^{dc}
$D_4B_1K_1$	71.3^{bc}	62.7^{cd}	82.0^{bc}	80.0^{b}	91.0^{a}	80.0^{b}	82.0^{bc}	70.7^{cd}	71.0^{b}
$D_4N_1B_1$	62.0^{bcd}	81.0^{ab}	72.3^{dc}	69.3^{bc}	44.0^{d}	61.3^{c}	72.3^{cd}	89.7^{b}	50.7^{cd}
D_5K_1	52.0^{cd}	72.7^{bc}	62.7^{d}	61.3^{c}	60.7^{bc}	91.3^{a}	80.0^{bc}	97.3^{a}	71.3^{b}
N_4	42.0^{d}	60.0^{d}	61.3^{d}	80.7^{b}	52.3^{cd}	70.0^{bc}	69.3^{cd}	51.3^{e}	31.0^{e}
D_8	71.3^{bc}	64.0^{cd}	89.7^{ab}	80.3^{b}	50.7^{cd}	79.7^{b}	91.3^{ab}	51.0^{e}	63.0^{bc}
D_4N_1	81.3^{b}	89.0^{a}	81.7^{bc}	60.0^{c}	61.0^{bc}	92.0^{a}	59.3^{d}	66.3^{cde}	53.3^{cd}
D_4N_2	61.7^{bcd}	63.0^{cd}	91.3^{a}	80.0^{b}	62.7^{bc}	71.0^{bc}	70.0^{cd}	66.3^{cde}	51.3^{cd}

Table 4
Time period for 85% and above callus induction.

Media code	DBW-14	DBW-17	DBW- 38	DBW-39	HD-2894	HD-2967	HD-2733	HD-2932	HD-2987
$B_{1.5}$	5^{d}	9^{ab}	8^{abc}	10^{a}	9^{ab}	8^{a}	6^{b}	9^{ab}	7^{ab}
B_2	10^{a}	9^{a}	8^{bc}	6^{dc}	7^{bc}	8^{a}	6^{b}	7^{c}	7^{b}
D_4B_1	7^{bc}	7^{c}	8^{c}	6^{d}	9^{a}	8^{a}	8^{a}	7^{c}	9^{a}
$D_4B_1K_1$	9^{ab}	9^{a}	10^{a}	6^{d}	5^{d}	8^{a}	8^{a}	7^{bc}	7^{b}
$D_4N_1B_1$	9^{abc}	7^{bc}	10^{ab}	8^{bc}	7^{c}	8^{a}	8^{a}	7^{c}	7^{b}
D_5K_1	9^{ab}	7^{c}	10^{a}	8^{ab}	9^{a}	6^{b}	8^{a}	5^{d}	7^{b}
N_4	9^{ab}	9^{a}	10^{ab}	8^{abc}	9^{a}	8^{a}	8^{a}	9^{a}	9^{a}
D_8	10^{a}	9^{a}	8^{c}	8^{bc}	7^{c}	8^{a}	8^{a}	7^{c}	7^{b}
D_4N_1	9^{ab}	10^{a}	8^{bc}	8^{abc}	9^{a}	8^{a}	8^{a}	9^{a}	7^{b}
D_4N_2	7^{cd}	7^{c}	8^{bc}	8^{bc}	9^{a}	8^{a}	8^{a}	7^{c}	9^{a}

Table 5
Percent of callus growth and development after 3 weeks of incubation

Media code	DBW-14	DBW-17	DBW- 38	DBW-39	HD-2894	HD-2967	HD-2733	HD-2932	HD-2987
D_4B_1	32.3^{f}	21.3^{e}	29.7^{c}	43.0^{cd}	22.0^{c}	42.7^{d}	21.0^{d}	23.0^{d}	41.7^{c}
$D_4B_1K_1$	42.3^{ef}	51.3^{d}	44.0^{bc}	32.7^{d}	32.0^{c}	21.7^{e}	32.0^{d}	22.7^{d}	54.3^{bc}
$D_4N_1B_1$	51.3^{dc}	21.3^{e}	39.7^{bc}	40.0^{cd}	19.0^{c}	29.7^{ed}	24.3^{d}	13.0^{d}	50.7^{c}
D_5K_1	70.7^{bc}	53.3^{d}	52.7^{b}	71.0^{b}	62.0^{b}	72.7^{bc}	71.0^{c}	90.0^{a}	92.0^{a}
N_4	60.3^{cd}	70.7^{c}	49.0^{bc}	62.0^{bc}	50.3^{b}	60.7^{c}	79.3^{abc}	61.3^{c}	73.3^{b}
D_8	70.7^{bc}	60.3^{cd}	89.3^{a}	80.0^{b}	81.3^{a}	61.3^{c}	90.0^{ab}	72.0^{bc}	93.3^{a}
D_4N_1	80.3^{b}	90.0^{a}	88.0^{a}	97.3^{a}	81.3^{a}	82.3^{ab}	91.3^{a}	91.0^{a}	95.0^{a}
D_4N_2	97.0^{a}	81.0^{b}	93.7^{a}	96.0^{a}	80.3^{a}	89.7^{a}	79.0^{bc}	81.3^{ab}	92.0^{a}

Table 6
Variation of callus size in of Indian wheat genotypes nurtured in different media

Media code	DBW-14	DBW-17	DBW- 38	DBW-39	HD-2894	HD-2967	HD-2733	HD-2932	HD-2987
D_4B_1	1.7^{b}	2.0^{c}	3.3^{b}	4.3^{bc}	0.7^{b}	1.7^{c}	3.7^{bc}	3.0^{bc}	2.7^{bc}
$D_4B_1K_1$	1.3^{b}	1.0^{d}	2.7^{b}	3.0^{c}	1.0^{b}	3.0^{bc}	5.3^{a}	2.3^{c}	0.7^{d}
$D_4N_1B_1$	2.0^{b}	1.7^{cd}	4.3^{ab}	3.0^{c}	0.3^{b}	2.7^{bc}	2.7^{c}	2.7^{c}	2.3^{c}
D_5K_1	5.3^{a}	5.3^{ab}	4.0^{ab}	5.3^{ab}	5.0^{a}	3.7^{ab}	5.7^{a}	4.7^{b}	5.0^{abc}
N_4	5.0^{a}	4.3^{b}	4.0^{ab}	4.7^{bc}	4.3^{a}	4.3^{ab}	3.7^{bc}	7.0^{a}	5.0^{abc}
D_8	5.0^{a}	4.7^{ab}	5.3^{a}	4.0^{bc}	4.7^{a}	4.0^{ab}	4.7^{ab}	3.3^{bc}	4.3^{abc}
D_4N_1	4.7^{a}	6.3^{a}	5.7^{a}	7.7^{a}	5.0^{a}	5.3^{a}	6.3^{a}	4.3^{b}	6.3^{a}
D_4N_2	5.7^{a}	5.0^{ab}	4.3^{ab}	7.0^{a}	7.3^{a}	5.0^{a}	5.3^{a}	3.0^{bc}	5.3^{ab}

Table 7
Percent response of callus for shoot regeneration of different Indian wheat genotypes nurtured in shooting media

Media code	DBW-14	DBW-17	DBW- 38	DBW-39	HD-2894	HD-2967	HD-2733	HD-2932	HD-2987
B_2	12.8^{b}	55.0^{a}	28.6^{b}	14.3^{c}	30.6^{a}	61.3^{ab}	80.9^{a}	47.4^{a}	0
$B_2K_{0.5}$	0	0	0	58.1^{a}	0	33.1^{c}	23.3^{b}	$24.5b^{c}$	0
B_2K_1	24.2^{a}	0	36.9^{b}	0	0	73.1^{a}	21.7^{b}	36.1^{b}	14.3^{a}
$K_2N_{0.5}B_{0.5}$	0	0	67.2^{a}	68.1^{a}	0	31.7^{c}	80.9^{a}	40.8^{a}	23.3^{a}
$B_{0.225}$+36M	14.3^{b}	0	0	33.1^{b}	0	59.0^{b}	13.5^{b}	20.6^{c}	0

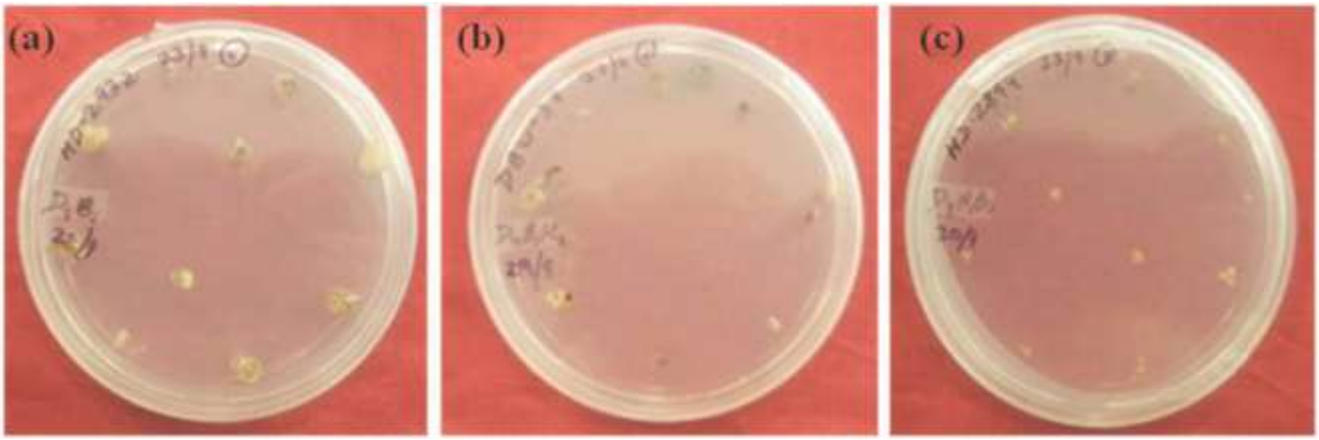

Fig. 2: Blackening of embryonal callus in different wheat cultivars on different media cv. HD 2932 on (a) MS + 2,4-D @ 4 mg/L + BAP @ 1 mg/L (b) cv. DBW 39 on MS + 2,4-D @ 4mg/L + BAP @ 1 mg/L + Kinetin @ 1mg/L (c) cv. HD 2894 on MS + 2,4-D @ 4 mg/L + NAA @ 1 mg/L + BAP @ 1mg/L

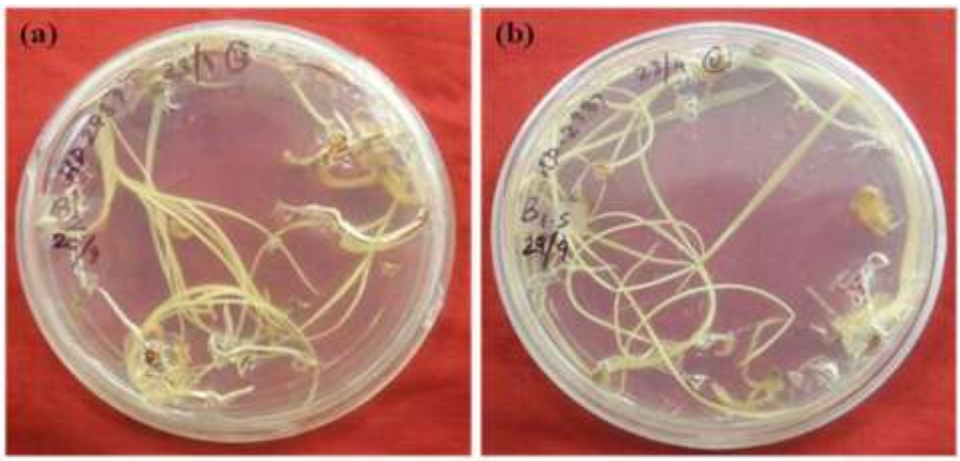

Fig. 3: Direct shoot induction in cv. HD 2987 on media (a) MS + BAP @ 2 mg/L and (b) MS + BAP@1.5mg/L

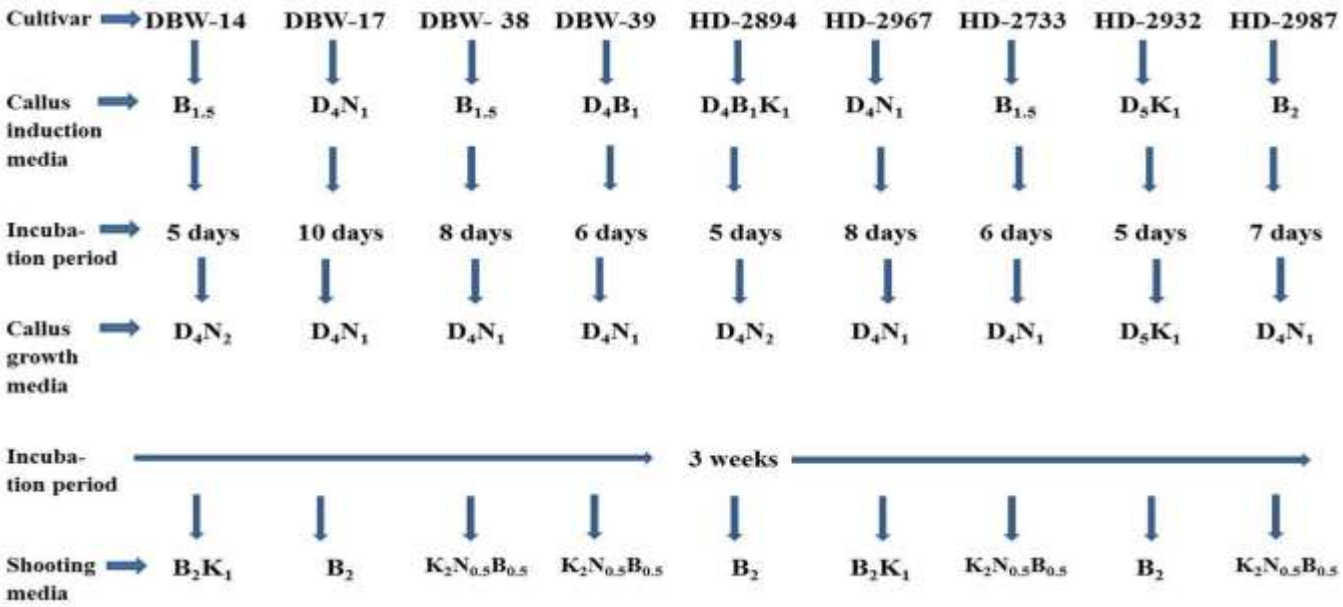

Fig. 4: Schematic representation of callus induction, growth and shoot regeneration from mature embryo in nine cultivars of India bread wheat in response to different culture media

Discussion

Choice of explant is the prime important criteria for successful cell and tissue culture protocols. Mature embryo is reported to be one of the good sources of primary explant for *in vitro* regeneration of wheat[35]. According to Özgen et al,[23] mature embryos have great advantage to be used as explants for wheat tissue culture, as it has high potentiality for callus induction and regeneration. Availability of mature embryo throughout the year availability is an added advantage. In this study, we have used mature embryos of nine Indian elite wheat cultivars and successfully produced calli from mature embryos.

Callus development and plant regeneration are the important steps in potential cell and tissue culture for plant improvement deploying biotechnological techniques. The growth regulator auxin has an essential role in callus induction, but its adverse effect on plant regeneration reduces efficiency of shoot regeneration culture media[27]. In this study, we have used 2,4-D and NAA as an auxin source. 2,4-D is not only one of the best growth regulators used for somatic embryogenesis[3] but studies have shown that it has been very effective for callus development and tissue differentiation in cereals, either when used alone or in combination with NAA, or both NAA and BAP, or with Kn[3,7,14,33]. We have used similar media for callus induction and development. Beside the media already reported by previous workers, we also formulated media with different concentration of NAA, 2,4-D, BAP and Kn to standardize the best media for some of the elite Indian wheat cultivars.

BAP was found to be best growth regulator for callus induction, although for further callus growth, BAP was not the suitable growth regulator. However, when the induced callus was transferred into the media supplemented with 2,4-D (4 mg/L) and NAA (1 mg or 2 mg/L), good callus growth and development were observed in all cultivars. Turhan and Baser[33] also reported the similar observations for a few winter wheat cultivars. Although, Sarkar and Biswas[29] reported that a higher concentration of 2,4-D (6 mg/L) gives the best result for callus induction in wheat with local wheat cultivars of Bangladesh, but Haliloglu[11] observed that 2,4-D @ 2 mg/L was optimum for regeneration of embryogenic callus in Bobwhite.

In our study with Indian cultivars, media with higher concentration of 2,4-D (6 mg/L) showed abortive growth of callus for most of the cultivars tested and no callus induction was observed in media supplemented with 2 mg/L 2,4-D thus confirming that cultivar depending factor seems to be operating in response of a particular concentrations of growth regulator in wheat under *in vitro* culture. However, there was no significant media specific response with respect to callus induction for all the cultivars after a period of ten days of incubation. Thus, media and cultivars influenced only early callus induction from mature embryos explants in wheat.

The callus developed in the media D_4N_1 and D_4N_2 were transferred to MS media supplemented with cytokinins and auxins for shoot regeneration. We have used BAP and kinetin as a source of cytokinin for *in vitro* shoot formation in wheat. Previously, it was shown that rice callus produced good shoot formation in some rice callus using BAP and kinetin in combination with NAA.[14] The similar media we had used in this study for regeneration of shoots from embryonic callus and we got maximum success in a few Indian cultivars (DBW 38, DBW 39, HD 2733, HD 2932 and HD 2987). Moreover, maximum response of shoot regeneration was observed in Indian cultivars: DBW 17, HD 2894, HD 2932 and DBW 14, HD 2967 on the media formulated in our study i.e. B_2 and B_2K_1. Keresa et al[16] used

hormone free MS media for shooting in Croatian cultivars of wheat, but success rate was 26% only, whereas Sarkar and Biswas[29] reported 30% regeneration in local wheat cultivar of Bangladesh. The media used by Hensel et al[12] were not much successful in shoot regeneration in Indian wheat cultivars.

Conclusion

The present study was successful in standardizing media for callus development and shoot regeneration *in vitro* for some Indian elite wheat cultivars (fig. 4). A further detailed study is needed to standardize the highly potent media for callus development and shoot regeneration in specific Indian elite wheat cultivars so as to develop transgenic wheat on a commercial scale.

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