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DOUBLED-HAPLOID PRODUCTION TECHNOLOGY IN WHEAT (*TRITICUM AESTIVUM* L.): ANTHER CULTURE

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ABSTRACT:

To achieve homozygosity within the shortest possible period, doubled-haploid technology is a valuable technique to reduce the time and cost of development of new wheat varieties. It involves *in vitro* development of fixed lines from the parental material; development of each generation of progeny can be initiated before the parents have achieved physiological maturity. The exploitation of recessive genes can be rapidly achieved by microspore culture and anther culture via induction of sporophytic pathway from a gametophytic pollen pathway, resulting in callus or embryoids formation. Anther culture studies were conducted to produce haploid plants in five bread wheat genotypes BW-2, BW-5, BW-8, BW-9, and BW-16. Two media liquid and agar-solidified with different compositions were used to determine their effects on the induction of calli and regeneration of green plants. The potential of callus induction was significantly higher (82.4) in liquid culture medium as compared to agar-solidified medium (55.7) worked out on 100 anther basis. In liquid media, calli induction ranged from 37.8% to 117%, whereas, calli induction ranged from 39.1 to 73.6% on agar-solidified medium. The callus induction and plant regeneration were varied among genotypes on both the media. Genotype BW-8 was more conducive for callus induction to agar medium as compared to liquid medium, however, BW-9 showed less callus differentiation ability on both the media.

INTRODUCTION:

Doubled-haploid technique is a unique method in plant breeding to achieve homozygosity and uniformity in the breeding lines within limited stunted time. Haploid plants are artificially regenerated from single microspores or macrospores with only one set of alleles on each locus. The di-haploidization often referred as doubled-haploid which doubles the haploid chromosomes and is regarded as the short cut method for obtaining homozygous breeding lines. Once the variability is induced in the breeding material, the plant breeder goes for his useful selections and is keenly interested in fixing that character avoiding any further undesirable segregation. In self-pollinated crops particularly cereals, doubled-haploid technique has been successfully employed for the improvement and many breeding programmes have been enriched by this technique **(**Zhou, 1989).

A number of haploid production methods have been investigated in crop species including microspore and anther culture (androgenesis), ovule culture (gynogenesis), *Hordeum bulbosum,* L. or maize (*Zea mays,* L.*)* pollination methods (alien species chromosome elimination), and alien cytoplasm system (Dunwell, 1985; Hu and Kasha, 1999). The production of doubled-haploid plants through anther/ microspore culture is relatively simple and a rapid route to complete homozygosity within short time and for expression of recessive genes

(Ouyang, 1986; Wei, 1982). It has a benefit of producing more than a thousand plants per cultured anther, as compared to all other methods, which limit to one haploid plant per floret (Devaux, 1988). Several factors have been identified important in the efficiency of wheat DH production. These include seasonal constraints, time of embryo excision, method of auxin application, and culture media composition. In the present studies, the efficiencies of callus induction and regeneration of green plants for wheat anther culture on liquid and agar-solidified media has been discussed.

MATERIALS AND METHODS:

Five bread wheat genotypes viz., BW-2 (Seri-82//Vee'S/Snbs),BW-5 (Snbs/ Clement/ Alds), BW-8 (Sham-4/76529//A5-3), BW-9 (EGVD-14/Roshan), BW-16 (KVZ/ Cgn//Sudan''4), developed through cross breeding programme at International Centre for Agriculture Research in the Dry Areas (ICARDA) Aleppo, Syria were used. The material was grown in plant growth chamber maintained at $20^0C \pm 2$ temperature during daytime and $15^0C \pm 2$ at night and humidity was 70-80%. The plant growth chamber was provided with 350 to 400 μmol m^{-2} S^{-2} of mixed fluorescent and incandescent light in a photoperiod of 16-h light per day. The plant growth period was about 8 weeks. Spikes from each genotype were collected before they emerged from flag leaf sheath. The sampled spikes were kept in a jar containing water and stored in refrigerator for cold treatment in the darkness at 4^0C for 7 days. After cold treatment for 4 days, the spikes were twice surface-sterilised with a disinfectant chlorox (4%) for 5 minutes and washed thoroughly with distilled water 3 times for 5 minutes. Anthers were removed from sheath with a fine sterilised forceps, squashed in a drop of acetocarmin (1-2% carmine ± 45% acetic acid) and stained on glass slide and examined under microscope to see the appropriate stage. The anthers having mid-late uni-nucleate microspore stage were plated aseptically on induction medium.

For the calli induction, two media were used: i) A basic CHB-3 medium containing kinetin (0.5 mg/L), 1.2 mg/L NAA (1-naphtheleneceticacid), 0.5mg/L glutamine and 0.5mg 2-4D were used. Ii) Liquid (without gelling agent), agar (5g/L),ficoll-supplemented (100mg/L) media was also used as an induction medium. In each liquid culture or agar culture, 15ml liquid or agar medium were used. Anthers were directly inoculated on each petridish and were gently sealed with para film and incubated at $28^0C \pm 1$ under dark conditions. After 30-35 days when embryoides/calli had grown 1-2mm in diameter were transferred aseptically to agar-solidified 190-2-regeneration medium (Zhuang and Xu 1983) supplemented with 0.5mg/L KT or 5mg/L indolebutyricacid (IBA), 0.5mg/L NAA and 0.1 mg/LGA_3 (gibberellic acid). Petridishes (100mm x 15mm) containing 20-25 calli were incubated at room temperature $22\text{-}25^0C$ under continuous fluorescent lights in a 16-h photoperiod. Data were recorded on number of anthers plated, number of calli induced, callus induction percent, number of calli plated, number of calli regenerated, plant regeneration percent, number of green and albino plants regenerated and regeneration of green plants (%). After 20-30 days the green seedlings were transferred to plantlet (PLL) medium *in vitro* conditions, and kept them in the culture room under optimum conditions ($22\text{-}25^OC$) for one month at 16-h light. Then the plants were transferred to peat soil and incubated at the controlled environment $12\text{-}15^OC$ for a week then $18\text{-}20^OC$ for 3 weeks. Stable

plants from growth room with better growth were up-rooted and the roots were treated with colchicines 0.2% for 4 hours. Washed the roots with distilled water for 24 hours and then again replanted the doubled-haploids in the soil. The present paper will be focussed on induction and regeneration of calli/plants on two liquid and agar-solidified media.

RESULTS AND DISCUSSIONS:

The response of wheat genotypes for callus induction from anthers grown on liquid and agar-solidified media is given in Table 1. All the five genotypes showed differential response for anther culture on both liquid and agar-solidified media. The average callus induction per 100 anthers in liquid culture medium (82.4) was significantly higher as compared to agar-solidified medium (55.7). In liquid medium, number of calli produced per 100 anthers ranged from 38 in genotype BW-9 to 117 in BW-8; whereas, on agar-solidified medium, the number ranged from 39 in BW-8 to 74 in BW-5. Except BW-9, the other four genotypes possessed better potential for producing calli on liquid medium. On agar-solidified medium, the rate of producing calli was higher in two genotypes BW-2 and BW-5 as compared to other genotypes. The genotype BW-8 was found to be more conducive for the production of calli on liquid medium and showed the minimum response under agar-solidified medium. It was reverse in case of BW-9, where relatively better response was found under the agar medium. Almost similar response was noted in BW-5 in both the media. The liquid culture of anthers /microspores has also been exploited for different crop species including wheat (Tian and Chen, 19834: Lazer *et al*., 1985; Wei *et al*., 1986). Wheat genotypes showed significant differences in response to regeneration of plants on liquid and agar media (Tables 2 and 3). The regeneration percent of plants derived from calli on liquid medium (43%) was more than double as compared to agar media (18.5%). The percent regeneration of green plants was comparable in both the media, which was 66.8% on agar-solidified media and 62.4% on liquid media. Similar trend was found for the albino plants on liquid medium as well as on after medium. Jones and Petiano (1988) in wheat also reported that the regeneration ability of calli and the ratio of green to albino plants in liquid culture were lower than that of agar culture. Genotype BW-2 showed more ability of regeneration of plants (66%) and genotype BW-8 the least (24.2%) on liquid medium (Table 2). Likewise, the maximum regeneration of plants on agar-solidified medium occurred in genotype B.W-16 (28.4%) and the minimum (11.3%) in B.W-

5. In the genotype BW-8, although the callus induction was the highest (117 on 100 anthers) in liquid medium gave the poorest response for regeneration and % of green plants. This situation has been quite reverse on the agar-solidified medium where with minimum calli induction a better response of green plant has been observed. In the comparative performance, genotype BW-16 produced lowest number of calli (40) on agar-solidified medium with only 39.4% green plants (Table 3).

Although no particular Mendalian ratio has been observed in the pattern of green and albino plants on either media, yet it can be safely concluded that except genotype BW-8 in liquid medium and BW-16 in agar-solidified medium, all other genotypes had responded positively for the production of green plantlets. There are enough possibilities of achieving good number of doubled-haploid plants genetically homozygous through this technique.

Table 1. Induction of callus on liquid medium and agar-solidified medium initiated from wheat anthers.

Genotypes	Liquid medium			Agar-solidified medium		
	Total no. of anthers plated	Calli induced	Calli induced per 100 anthers	Total no. of anthers plated	Calli induced	Calli induced per 100 anthers
BW-2	450	433	96	278	196	70
BW-5	380	285	75	330	243	74
BW-8	255	298	117	245	96	39
BW-9	645	244	38	457	252	55
BW-16	365	315	86	317	128	40
Mean	419	315	82.4	325	183	55.7

Table 2. Regeneration of plants from anther-derived wheat calli induced on liquid medium.

Genotypes	Total no. of calli plated	Total no. of plants regenerated	%plants regenerated	No of green plants	No albino plants	% green plants
BW-2	150	99	66.0	65	34	65.7
BW-5	210	73	34.7	55	18	75.3
BW-8	95	23	24.2	9	14	39.1
BW-9	204	112	54.9	79	33	70.5
BW-16	176	65	36.9	40	25	61.5
Mean	167.0	74.4	43.3	49.6	24.8	62.4

Table 3. Regeneration of plants from anther-derived wheat calli induced on gar-solidified medium.

Genotypes	Total no. of calli plated	Total no. of plants regenerated	% plants regenerated	No of green plants	No. of albino plants	% green plants
BW-2	195	41	21.0	24	17	58.5
BW-5	203	23	11.3	18	5	78.2
BW-8	44	8	18.2	6	2	75.0
BW-9	88	12	13.6	10	2	83.0
BW-16	116	33	28.4	13	20	39.4
Mean	129.2	23.4	18.5	14.2	9.2	66.8

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