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Research note

Callus induction and plant regeneration from mature embryos of a diverse set of wheat genotypes

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Abstract

This paper compared the behavior of a diverse set of wheat genotypes in their tissue culture response. Significant differences were detected in plant regeneration, culture efficiency, and regeneration capacity when mature embryos of 47 wheat cultivars, breeding lines, and the common wheat progenitors, *Triticum monococcum*, *T. tauschii,* and *Aegilops speltoides* were compared. Although not currently used in wheat tissue culture, mature embryo-derived callus of cv. 'Zak' (SWS), 'Scarlet' (HRS), 'Tara' (SWS), 'Jagger' (HRW), 'UC 1036' (HRS), and 'Kyle' durum showed better or comparable plant regeneration than commonly cultured cultivars 'Fielder' and 'Bobwhite.' Of the three diploid wheat progenitors tested, *Ae. speltoides* regenerated the most plants. In one replicated experiment, callus induction was correlated with culture efficiency ($r = 0.42$; $p = 0.002$) and regeneration capacity ($r = 0.39$; $p = 0.002$), and in a second larger screen, callus induction correlated with the total number of plants regenerated ($r = 0.6$; $p = 0.001$). Immature and mature embryos of 'Bobwhite' and 'Crocus' were compared for callus induction and plant regeneration. Immature embryos were superior explants in terms of plant regeneration. However, sufficient numbers of plants can be regenerated from mature embryos saving on growth facility resources and time required for the collection of immature embryos.

Abbreviations: SWS – soft white spring; HRS – hard red spring; HRW – hard red winter; HWS – hard white spring

Two of the many factors limiting hexaploid wheat transformation using particle bombardment or *Agrobacterium* infiltration of embryogenic tissue are the long time periods required to produce transformants, and the inferior quality and agronomic traits of the recipient cultivars. Previous research has demonstrated immature wheat embryos to be an excellent source of explants (Ahloowalia, 1982; Ozias-Akins and Vasil, 1982; Sears and Deckard, 1982). 'Bobwhite' (Weeks et al., 1993) and 'Fielder' (Nehra et al., 1994) have become the standard cultivars used in bioengineering and tissue culture because plants are consistently regenerated. However, these cultivars do not possess superior agronomic or quality traits. Mature embryos from seeds can be used directly in tissue culture (Ozias-Akins and Vasil, 1983; Özgen et al., 1998; Delporte et al., 2001). Explants derived from mature wheat endosperm supported embryos (Özgen et al., 1998) and thin mature embryo fragments (Delporte et al., 2001) were used for callus induction and plant regeneration. Use of mature embryos saves time and space, and reduces greenhouse costs associated with growing plants to post-anthesis for the collection of immature embryos. Winter wheat research requires the additional input of controlled low temperature chambers to satisfy the vernalization requirements for floral induction prior to embryo collection. However, it is not known which elite cultivars

optimally induce callus and regenerate plants from mature embryos.

The purpose of this research was to screen a diverse set of high quality, agronomically acceptable spring and winter wheat cultivars and breeding lines of all market classes for their ability to induce callus and regenerate plants from mature seed embryos dissected free of the endosperm. Three accessions of the wheat progenitors (*T. monococcum, T. tauschii*, and *Aegilops speltoides*) also were examined because they serve as diploid models for wheat genetic research. In addition, mature and immature embryos of 'Bobwhite' and 'Crocus' were compared for their ability to produce callus and regenerate plants.

Forty-seven different genotypes of wheat were examined for callus induction and plant regeneration. Seeds of all market classes of hexaploid spring wheat cultivars and breeding lines (WA) developed in the U.S. Pacific Northwest region were obtained from Dr. Kimberlee Kidwell, Washington State University. Seed of hard red winter wheat cultivars from the Midwest, 'Karl' and 'Jagger,' were supplied by Dr Allan Fritz, Kansas State University. Southwestern breeding line 'UC 1036' was acquired from Dr. Jorge Dubcovsky, UC Davis. 'Neeley', 'Tam 106' and wheat progenitors were obtained from the USDA/ARS National Plant Germplasm System.

For the first experiment, 60 seeds of each genotype were washed in 70% ethanol for 10 min with shaking, surface sterilized in 30% bleach with 0.08% Tween 20 for 30 min at 22 ± 2 °C with shaking, and rinsed three times with double distilled water. In the second experiment, seed lots were surface sterilized with 70% bleach with 0.08% Tween 20 for 30 min to decrease problems with fungal contamination with no detrimental effect on the seed. The seeds were left in the last rinse for two h to imbibe water and soften the endosperm prior to embryo dissection. Embryos were removed aseptically from the endosperm, bisected longitudinally, and plated with scutella up on callus induction media (CIM) pH 5.8 (MS media [Sigma M5524] supplemented with 30 g l^{-1} maltose, 0.25 g l^{-1} *myo*-inositol, 1.0 mg l^{-1} thiamine-HCL, 1.0 g l^{-1} casein hydrosylate, 2.5 mg l^{-1} dicamba and 0.69 g l^{-1} L-proline solidified by 3.5 g l^{-1} phytagel) in the dark at 22 ± 2 °C (Horvath et al., 2001).

The media and culture protocols described by Horvath et al. (2001) were employed. Callus was induced on CIM for 54 d, changing to fresh plates every 14 d. During each transfer, all roots and shoots were removed from the calluses to ensure that the regenerating shoots were not derived from existing meristematic tissue. On day 54, all calluses were transferred to CIM Green (CIM with 0.1 mg l^{-1} 6-benzyl-aminopurine). The calluses were placed in a 16 h light/8 h dark cycle at 22 ± 2 °C for 14 d. They were transferred to shoot growth media (SGM) pH 5.6 (MS [Sigma M2909] with 165 mg l^{-1} ammonium nitrate, 62 g l^{-1} maltose, 0.1 g l^{-1} *myo*-inositol, 0.4 g l^{-1} thiamine-HCl, 1 g l^{-1} casein hydrosylate, 1 mg l^{-1} 6-benzyl-aminopurine, 0.75 g l^{-1} glutamine solidified with 3.5 g l^{-1} phytagel) for 30 d, changing to fresh plates after 14 d. On day 93, the calluses were transferred to phytatrays (Sigma) containing root growth media (RGM) (CIM without dicamba) and grown for 16 d after which time they were scored.

In the first experiment, mature embryos from a diverse set of 18 wheat genotypes and wheat progenitors were tested for callus induction and plant regeneration. Twenty half-embryos were plated on culture media and grown in a randomized complete block with three replications. The variables for this experiment included mean callus induction, mean number of green plants (with roots and shoots) regenerated, regeneration capacity (the mean number of plants regenerated/calluses induced × 100), and culture efficiency (the mean percentage of plants regenerated/half embryos plated × 100). Count data for the calculated variables were not normally distributed, therefore, a square root transformation was performed on the variable number of plants regenerated and a log10 transformation on the percentage data (Steele and Torrie, 1980). Two-way ANOVAs and LSD comparisons (Steele and Torrie, 1980) were performed among the 18 cultivars. Callus induction was highest in 'Fielder,' 'Crocus,' and '*T. monococcum*' and the lowest in 'Wawawai,' 'Neeley,' 'Chinese Spring' and 'Kyle' durum (Table 1). Thirty percent of the calluses in most cultivars were embryogenic and regenerating plants by day 68. Significant cultivar differences were detected for 'the number of plants regenerated' ($p = 0.019$) and LSD multiple comparisons produced two statistical groups (Table 1; LSD ±2.4). 'Fielder,' 'Zak,' 'Scarlet,' 'Tara,' 'UC 1036,' 'Jagger,' 'Bobwhite,' 'Kyle,' 'Chinese Spring,' and *Aegilops speltoides* regenerated the most plants. Although there were genotype differences in 'regeneration capacity' ($p = 0.026$), most of the genotypes fell into the uppermost statistical group (Table 1) indicating similarity in the number of plants produced relative to calli induced. Multiple comparisons of the variable 'culture efficiency' grouped 'Fielder,' 'Zak,' 'Tara,' 'UC 1036,'

Table 1. Callus induction and plant regeneration from mature embryos of 18 *Triticum* members

Triticum member[1]	Genome	Class	Origin[2]	Callus Induction[3]		SQRT (number of plants reg)[4]		Number of plants reg[5]	Regeneration capacity[6]			Culture efficiency[7]		
				Mean	± SE	Mean♦	± SE	Mean	Mean♦	± SE	Antilog of Mean	Mean♦	± SE	Antilog of Mean
Fielder	ABD	SWS	NW	20	0.0	3.0 a	0.2	9.0	1.6 a	0.1	45	1.6 a	0.1	45
Wawawai	ABD	SWS	NW	13	6.5	2.2 b	1.2	7.3	1.1 b	0.6	37	1.1 b	0.6	37
Zak	ABD	SWS	NW	18	0.0	4.6 a	1.9	28.7	1.9 a	0.3	159	1.9 a	0.3	143
Scarlet	ABD	HRS	NW	19	0.3	2.7 a	0.2	7.3	1.6 a	0.1	38	1.5 b	0.1	37
Tara	ABD	HRS	NW	19	0.6	3.7 a	0.3	14.0	1.9 a	0.1	73	1.8 a	0.1	70
Neeley	ABD	HRW	NW	13	6.7	1.2 b	0.6	2.3	0.8 b	0.4	12	0.8 b	0.4	12
UC 1036	ABD	HWS	SW	19	1.3	3.9 a	0.7	16.3	1.9 a	0.1	85	1.9 a	0.2	82
Karl	ABD	HRW	MW	18	0.6	2.0 b	0.3	4.0	1.3 a	0.1	22	1.2 b	0.1	20
Jagger	ABD	HRW	MW	18	1.5	4.7 a	0.3	22.0	2.1 a	0.1	126	2.0 a	0.1	110
Tam106	ABD	HRW	S	19	0.6	1.2 b	0.2	1.7	0.9 b	0.2	9	0.8 b	0.2	8
Bobwhite	ABD	HWS	Mexico	19	0.0	3.3 a	0.7	12.0[8]	1.7 a	0.2	63	1.7 a	0.2	60
Crocus	ABD	HRS	Canada	20	0.3	1.5 b	0.1	2.3[9]	1.1 b	0.1	12	1.0 b	0.1	12
Columbus	ABD	HRS	Canada	19	0.3	2.2 b	0.5	5.7	1.4 a	0.2	30	1.4 b	0.2	28
Kyle	AB	durum	Canada	11	5.7	4.5 a	2.3	31.0	1.6 a	0.8	184	1.6 a	0.8	155
Chinese Spring	ABD	–	China	13	1.5	4.1 a	0.8	18.0	2.1 a	0.2	151	1.9 a	0.2	90
T. tauschii	D	–	Iran	16	1.4	1.6 b	0.1	2.7	1.6 a	0.2	51	1.1 b	0.1	13
T. monococcum	A	–	Macedonia	20	0.3	0.7 b	0.7	1.7	0.5 b	0.5	8	0.5 b	0.5	8
Ae. speltoides	B	–	Turkey	14	0.9	2.6 a	0.7	7.7	1.6 a	0.2	55	1.5 b	0.2	38
Mean ± SE				**17** ±	**0.6**	**2.8** ±	**0.2**	**11.0**	**1.5** ±	**0.1**	**64.0**	**1.4** ±	**0.1**	**54.0**
LSD $_{0.05}$ ±				**ns**		**2.4**		–	**0.9**		–	**0.4**		–

[1]For pedigree information, USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)*. http://www.ars-grin.gov/cgi-bin/npgs/html/csr.pl.
[2]Origin is USA unless otherwise indicated. NW=Northwest; N=North; MW=Midwest; W=West; S=South.
[3]Mean callus induction is the average number of calli induced over three replicates of 20 half embryos plated.
[4]Square root transformation of (average number of plants regenerated).
[5]Average number of green plants regenerated from three replicates.
[6]Regeneration capacity = (Average number of green plants regenerated/ induced calli) × 100. Data did not fit normal distribution and were transformed with the log10 transformation and ANOVA performed on transformed values. Transformed values were converted back to percentage data with the antilog function.
[7]Culture efficiency = (Average number of green plants regenerated/20 half embryos plated over three replicates) × 100. Data did not fit normal distribution and were transformed with the log10 transformation and ANOVA performed on transformed values. Transformed values were converted back to percentage data with the antilog function.
[8]The mean number of plants regenerated from immatue embryos of Bobwhite (58.0± 0.9).
[9]The mean number of plants regenerated friom immature embryos of Crocus (9.3± 2.9).
♦Means followed by the same letter are not significantly different at the 5% level of significance.

'Jagger,' 'Bobwhite,' 'Kyle,' and 'Chinese Spring' into a similar statistical group (LSD ±0.4) indicating similarity in the number of plants produced relative to the number of embryos plated. Callus induction was significantly correlated with culture efficiency ($r = 0.42$; $p = 0.002$). There was less of a correlation between callus induction and regeneration capacity of the callus ($r = 0.39$; $p = 0.005$), and no correlation between callus induction and the 'square root transformed values of the number of plants produced.' As an example of the latter, an inverse relationship between callus induction and plant regeneration in the progenitor accessions (Table 1) was detected. Of the wheat progenitors, *Ae. speltoides* regenerated the most plants.

Immature and mature embryos of 'Bobwhite' and 'Crocus' were compared for tissue culture performance. The immature embryos were collected 14 days post-anthesis and three replicates of 20 half-embryos per replicate were cultured using the protocol described for mature embryos. When 'Bobwhite' callus was derived from immature embryos an average of 58

Table 2. Callus induction and plant regeneration from mature embryos of 29 hexaploid wheat cultivars and lines

Cultivar/ Line[1]	Origin[2]	No of half embryos plated	Total callus induction	Total no of plants regenerated	Regeneration capacity[3]	Culture efficiency[4]
Alpowa	NW	60	17	3	18	5
Hank	MW	60	39	14	36	23
Edwall	NW	60	37	17	46	28
Penawawa	NW	20	18	5	28	25
Calorwa	NW	60	20	18	90	30
Winsome	NW	20	19	6	32	30
Butte86	MW	40	37	10	27	25
Challis	MW	40	32	5	16	12
Maron	NW	60	59	11	19	18
Eden	NW	60	54	18	33	30
WPB926	MW	60	60	50	83	83
WA7839	NW	20	18	16	89	80
WA7859	NW	60	60	24	40	40
WA7860	NW	60	50	30	60	50
WA7875	NW	60	48	24	50	40
WA7877	NW	40	38	3	8	8
WA7883	NW	60	40	14	35	23
WA7884	NW	60	20	5	25	8
WA7886	NW	60	60	17	28	28
WA7887	NW	40	40	17	42	42
WA7890	NW	20	16	7	44	35
WA7892	NW	60	58	49	84	82
WA7893	NW	60	37	19	51	32
WA7900	NW	60	58	21	36	35
WA7901	NW	60	50	14	28	23
WA7904	NW	60	38	6	16	10
WA7905	NW	40	38	5	13	12
WA7914	NW	60	58	14	24	23
WA7915	NW	60	55	15	27	25
Mean		**51**	**40**	**16**	**39**	**31**
Std Dev ±		**15**	**15**	**12**	**23**	**21**

[1]All of these wheat lines originated in US.
[2]NW=Northwest; MW=Midwest. WA numbers are Washington State University numbers. WPB is the Western Plant Breeder's designation.
[3]Regeneration capacity = (total number of plants regenerated/embryos induced) × 100.
[4]Culture efficiency = (total number of plants regenerated /embryos plated) × 100.

± 0.9 plants were regenerated while an average of 12 + 5.0 plants were regenerated from mature embryo-derived callus. Similarly, immature embryo-derived 'Crocus' callus regenerated an average of 9.3 ± 2.9 plants, compared to an average of 2.3 + 0.3 from mature embryo-derived callus. Mature embryos-derived calluses were smaller, darker, and readily regenerated roots compared with shoots (data not shown).

A larger set of 29 elite wheat cultivars and breeding lines was screened for callus induction and plant regeneration using mature embryos. The variables included the total number of embryos induced to form calluses, the total number of plants regenerated, regeneration capacity of the callus (total number of plants regenerated/calli induced × 100), and culture efficiency (the total number of plants regenerated/60

half embryos plated × 100). Total callus induction was highest in 'WPB926,' 'WA7859' and 'WA7886' (Table 2). The total number of plants regenerated was greatest from 'WPB926' and 'WA7892.' Total callus induction was significantly correlated with total number of plants regenerated ($r = 0.6$; $p = 0.001$). There was no significant correlation between total callus induction and 'regeneration capacity' or 'culture efficiency' indicating that these variables were not related.

This research has demonstrated that agronomically acceptable genotypes across all market classes can induce callus and regenerate plants from mature embryos at frequencies comparable to mature embryos of 'Bobwhite' and 'Fielder,' the standard cultivars used in bioengineering. Although these superior genotypes were not tested for transformation efficiency *per se*, the implications are that they may potentially be used in transformation work, and this may alleviate some of the difficulties associated with using non-adapted cultivars for genetic improvement. While immature embryos were a superior source of explant for regenerating large numbers of plants, mature embryos were an acceptable source, and thus can be used to save time and growth space required to grow plants to post-anthesis for embryo collection.

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