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CALLUS INDUCTION AND PLANT REGENERATION IN PROPAGATION OF WHEAT HYBRIDS WITH INTRODUCED A^M (*TRITICUM MONOCOCCUM*) OR R (*SECALE CEREALE*) GENOME

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This study assessed the regeneration potential of interspecific and intergeneric wheat hybrids with the A^m genome of diploid wheat (*T. monococcum*) introduced, in comparison to wheat hybrids with the R genome of rye, in the propagation of F_1 hybrids in vitro. Tetraploid hybrids with the AA^mBD and ABDR genotype formed callus tissue from rachides with significantly higher frequency than from peduncles. The exception was the triploid A^mDR genotype, which formed callus from rachides and peduncles with similar frequency. Callus with the AA^mBD genotype showed the highest regeneration ability (37.1%), and the ABDR genotype the lowest (6.2%). Plant regeneration from peduncle explants was twice more frequent than from rachis explants. Plant regeneration from peduncles and rachides of the A^mDR F_1 hybrid was intermediate (12.3%). The ability of young regene-rants to start independent development was the most important factor determining the effectiveness of in vitro propagation. Multiplication of callus and plant regeneration was shown to be a promising method of the maintaining and vegetatively propagating the hybrids AA^mBD and A^mDR expressing strong incompatibility barriers.

Key words: *Triticum monococcum, Secale cereale, T. aestivum*, immature inflorescence, callus culture, plant regeneration.

INTRODUCTION

Wheat interspecific and intergeneric experimental hybrids with the introduced A^m genome of the diploid species *T. monococcum* were obtained via in vitro culture of immature zygotic embryos (Sodkiewicz et al., 2001). Pre- and postzygotic barriers expressed in these cross combinations largely limit the ability to obtain satisfactory numbers of plants, and they reduce the number of F_1 hybrid plants with AA^mBD and A^mDR genotypes to only a few. In spite of effective integration of the A^m genome with other genomes inside the AA^mBD hybrid, it remains totally sterile, with sterile anthers and also disturbances of ovary development, making generative plant reproduction impossible. The triploid A^mDR hybrid is the result of crossing *T. monococcum* with rye and *T. tauschii* – being the new hexaploid triticale parent – but development of plants with this genotype is also limited because of physiological disturbances in the hybrid plant. Micropropagation, the most common technique used in this biotechnology area at the moment, is a useful method of in vitro vegetative multiplication of F_1 hybrids (Nakamura et al., 1981; Fedak, 1985; Vasil et al., 1986; Bhaskaran et al., 1990; Wojciechowska and Pudelska, 1992). Plant regeneration on artificial media is used as an intermediate stage in the development of new experimental plant genotypes (Ozias-Akins and Vasil, 1982; Bommineni et al., 1996; Zimny et al., 1997; Li et al., 2000). This method opens up two practical applications: testing large numbers of individuals on a small surface in a relatively short time; and breeding over a long period of time, storing the regenerated plants in cultures in vitro and planting the regenerants later at the optimal time.

In this study we attempted to propagate cereal AA^mBD and A^mDR hybrid genotypes in vitro, with the aim of increasing the amount of plant material to be used in amphiploidization, gene expression tests and backcrosses.

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MATERIALS AND METHODS

The plant material included F_1 hybrids of *T. aestivum* cv. Igna × *T. monococcum* var. *nigricultum* (AA^mBD), *T. aestivum* (line 217) × *S. cereale* cv. Dańkowskie Złote (DZ) (ABDR), and *T. tauschii* (D98) × amphiploid *T. monococcum*/*S. cereale* (A^mDR) obtained from in vitro culture of zygotic embryos. The plant material was cultivated in a greenhouse at 22/15±2°C day/night under a 14 h photoperiod, monitored by computer. In order to standardize the conditions of plant development, the hybrids were cultivated at the same time in one climatic chamber and on the same soil substrate. Young immature inflorescences produced by F_1 hybrids isolated from tillers were taken still wrapped in their leaf sheaths. Hybrid ears were surface-sterilized in 5% calcium hypochlorite for 5 min and subsequently rinsed 3 or 4 times in sterile water.

The primary explants were peduncle and rachis fragments 3–5 mm in length, cultured on MS (Murashige and Skoog, 1962) supplemented with 2 mg/l dicamba for callus induction. Explants (~10 fragments per dish) were placed in 5 cm Petri dishes with 10 ml medium. The material was incubated in darkness at 24°C. Callus was transferred to MS medium containing 2 mg/l kinetin for plant regeneration and subcultured 2 or 3 times every 6 weeks. The callus tissue with regenerating shoots was transferred and exposed to sodium light (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) under a 16 h photoperiod at 22±2°C.

For better rooting, small green plants were placed on MS medium without phytohormone; after development of the root system they were transferred to potted soil and placed in the culture chamber at 19±2°C under sodium light (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a 16 h photoperiod.

The between-genotype differences in the number of explants forming callus, callus induction efficiency and regeneration efficiency were analyzed using the χ^2 contingency test.

RESULTS

Of the 1393 explants cultured, the biggest shares were of the A^mDR (52.3%) and ABDR (42.6%) genotypes. The very low spike production of the AA^mBD genotype severely limited the number of explants obtained; they constituted only 5.1% of the total. Callus tissue initiation began 6 or 7 days after the explants were placed on the medium, and then gradually increased. The smaller the initial explant, the smaller the callus aggregates and the slower their growth. After 6 weeks, the callusing explants from peduncle and rachis fragments were counted and compared with the initial number of explants (Tab. 1). Total callus induction frequencies ranged from 78.2% to 98.6% of the initial number of explants. The callus induction response of the explants was analyzed in order to understand the background of the expressed differences in callus frequency. Callus formation from peduncles showed no significant differences between genotypes ($\chi^2_{max.} = 0.119$, $p>0.05$), but there were such differences in the efficiency of callus induction from rachis explants.

Callus was produced by all of the cultured rachis explants in the F_1 hybrid of *T. aestivum* × *T. monococcum* (AA^mBD) (100%), and by nearly all of the rachides from the *T. aestivum* × *S. cereale* (ABDR) hybrid (94%). Explants from the *T. tauschii* × amphiploid *T. monococcum*/*S. cereale* (A^mDR) hybrid showed a distinctly lower frequency of callus formation (78%). The AA^mBD and ABDR genotypes formed callus tissue significantly more frequently from rachides than from peduncles $\chi^2 = 13.328^{**}$, $p<0.01$). The A^mDR genotype formed callus from rachides and peduncle with similar frequencies ($\chi^2 = 0.263$, $p>0.05$). The tetraploid AA^mBD and ABDR genotypes had not only significantly more frequent callus initiation from rachides than the triploid A^mDR genotype ($\chi^2 = 102.91^{**}$, $p<0.01$) but also significantly higher total callus induction efficiency during the whole experiment ($\chi^2 = 15.61^{**}$, $p<0.01$).

The first young regenerant appeared on callus derived from ABDR hybrid rachis explants as early as the second week of culture (still on callus initiation medium supplemented with dicamba). Other regenerants appeared successively on callus of all tested genotypes, but the highest number of plantlets was noted during weeks 4 and 5 of culture (Figs. 1, 2). Plant regeneration differed significantly between *T. aestivum* × *T. monococcum* (AA^mBD) and *T. aestivum* × *S. cereale* (ABDR) hybrids, despite their similar frequencies of callus induction (Tabs. 1, 2). The ABDR hybrid showed very low regeneration ability (6.2%), and the AA^mBD hybrid the highest (37.1%), with the *T. tauschii* × amphiploid *T. monococcum*/*S. cereale* (A^mDR) F_1 hybrid at an intermediate level (12.3%). Thus, regeneration ability appeared independent of callus formation efficiency.

The type of explant (rachis or peduncle) yielding maximum regeneration from callus depended on the cross and F_1 hybrid genotype. Callus obtained from rachides produced more morphogenic centers and regenerated plants in the *T. aestivum* × *T. monococcum* (AA^mBD) F_1 hybrid, whereas more regenerants were obtained from callus originating from the peduncle in the *T. aestivum* × *S. cereale* (ABDR) hybrid. Total plant regeneration frequency was significantly higher for peduncle callus than for rachis callus ($\chi^2 = 11.114^{**}$, $p<0.01$).

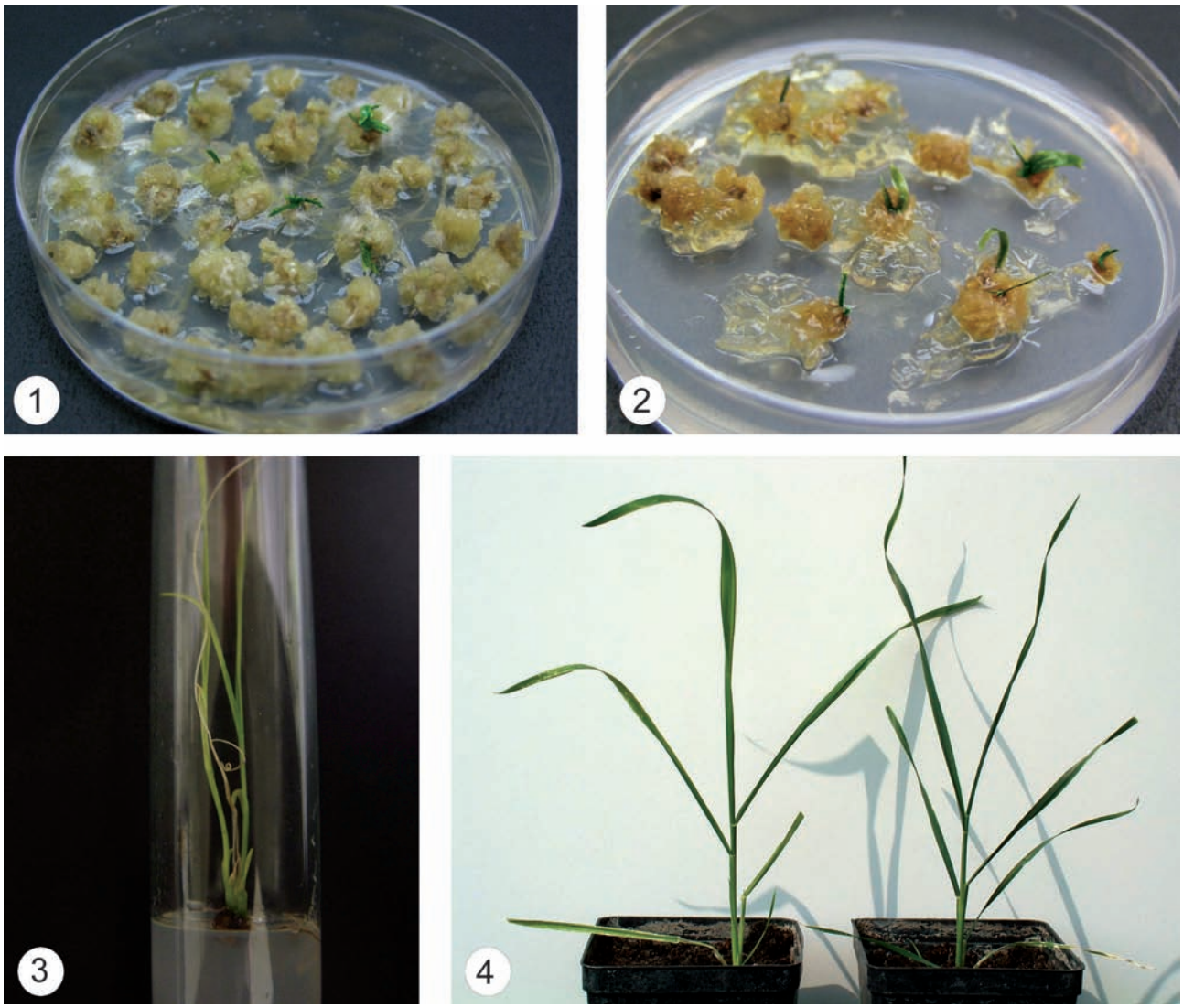

Fig. 1. Regeneration from rachis callus of *T. aestivum* cv. Igna × *T. monococcum* var. *nigricultum* hybrid.
Fig. 2. Regeneration of *T. tauschii* (D98) × amphiploid *T. monococcum*/*S. cereale* (Cd4/22) hybrids from peduncle callus.
Fig. 3. Young *T. aestivum* cv. Igna × *T. monococcum* var. *nigricultum* regenerants before development of root system.
Fig. 4. Rooted regenerants of *T. aestivum* cv. Igna × *T. monococcum* var. *nigricultum* hybrids.

The ability of young regenerants to start independent development turned out to be an important factor determining the number of developed plants. In this respect, the regenerants of $AA^{m}BD$ F_1 hybrids had the highest efficiency of culture, acclimating to greenhouse conditions best (Tab. 3, Figs. 3, 4). Regenerants of the *T. aestivum* × *S. cereale* (ABDR) hybrids also acclimated well; although their regeneration efficiency was lowest, almost 40% of the regenerants developed further.

The final low efficiency of the *T. tauschii* × amphiploid *T. monococcum*/*S. cereale* ($A^{m}DR$) hybrid culture (Tab. 3) was due to browning of tissue and withering of very young plants in the initial stage of their development. These degradation processes were synchronized in most regenerants with the $A^{m}DR$ genotype and proceeded to the second or third leaf stage; some of them, however, developed the highest number of spikes per plant after successfully rooting in soil (Tab. 3).

DISCUSSION

Intensive development of cereal tissue culture over recent decades has yielded a useful technique applied in genetics and plant breeding. Monocotyledon plants, including distant hybrids of

TABLE 1. Response to callus induction of peduncle and rachis explants of F_1 hybrids with introduced A^m, D and rye R subgenomes from diploid species

Cross combination (F_1 hybrid)	Genotype	Number of explants		Total number of explants	Number of explants forming callus (%)				Total number of explants forming callus	Total callus induction (%)
		peduncle	rachis		peduncle		rachis			
217 × DZ	ABDR	55	539	594	44	(80.0)	508	(94.2)	552	92.9
D98 × Cd4/22	A^mDR	55	673	728	44	(80.0)	525	(78.0)	569	78.2
Igna × Tm10	AA^{m}BD	6	65	71	5	(83.3)	65	(100)	70	98.6
total [average]		116	1277	1393	93	[80.2%]	1098	[86.0%]	1191	[85.5%]

TABLE 2. Efficiency of plant regeneration in callus culture of F_1 hybrids with introduced A^m, D and rye R subgenomes from diploid species

Cross combination (F_1 hybrid)	Genotype	Total number of explants	Total number of explants forming callus	Number of regenerating plants (%)		Total number of regenerants[1]	Regeneration efficiency[2] (%)
				peduncle	rachis		
217 × DZ	ABDR	594	552	13 (29.5)	21 (4.1)	34	6.2
D98 × Cd4/22	A^mDR	728	569	6 (13.4)	64 (12.2)	70	12.3
Igna × Tm10	AA^{m}BD	71	70	1 (20.0)	25 (38.5)	26	37.1
total [average]		1393	1191	20 [21.5%]	110 [10.0%]	130	[10.9%]

[1]versus number of explants forming callus; [2]number of calluses/number of regenerants

TABLE 3. Characteristics of regenerated plants of F_1 hybrids with introduced A^m, D and R subgenomes from diploid species

Cross combination (F_1 hybrid genotype)	Plants regenerated[1] (%)	Chromosome number	Number of spikes ($\bar{x}$)[2]	Number of flowers ($\bar{x}$)[2]	Number of flowers with correctly formed ovary (%)	Number of back-crossed flowers	Fertility after backcross pollination (%)
217 × DZ (ABDR)	13 (38.2)	28	47 (3.61)	1622 (124.8)	1622 (100.0)	479	22 (4.59)
D98 × Cd4/22 (A^mDR)	3 (4.3)	21	41 (13.7)	1617 (539.4)	1394 (86.2)	1047[3]	6 (0.57)
Igna × Tm10 (AA^{m}BD)	17 (65.4)	28	49 (2.87)	1418 (83.4)	546 (38.5)	406	3 (0.74)

[1]number of plants rooting in soil; [2]mean value per regenerant; [3]this genotype was pollinated by pollen of 8x *Triticale* (ABDR)

the *Poaceae* family, have been propagated vegetatively. Various kinds of explants have been used in studies of cereal micropropagation: young anthers, fragments of leaves, nodes, internodes, tiller and root apical meristems, cell suspensions, scutella, immature embryos and young inflorescences. The latter two are the most frequently used cereal explants (Ahuja et al., 1982; Fedak, 1985; Bommineni et al., 1996; Zimny et al., 1997; Özgen et al., 1998; Wojciechowska, 2005). It has been found that, in addition to culture conditions, the genotype and the type of explant can also strongly affect the explant proliferation frequency and callus tissue regeneration (Nakamura et al., 1981; Małuszyńska, 1994).

We assessed the micropropagation abilities of different cereal hybrids, using their rachides and peduncles. The tested hybrid genotypes exhibited similar levels of callus induction from peduncles, and the total callusing efficiency of peduncle explants was lower than that of rachis explants. There were distinct between-genotype differences in the results from the use of rachides as callus sources. All rachis explants of the *T. aestivum* ×

T. monococcum (AA^mBD) F_1 hybrid formed callus; the callus formation efficiency of the *T. tauschii* × amphiploid *T. monococcum*/*S. cereale* (A^mDR) hybrid was significantly lower. In *Aegilops-Secale* hybrids, Wojciechowska (1997) found that rachides of all tested genotypes had lower proliferation ability than peduncle explants and young inflorescences. In *H. vulgare* × *S. cereale* hybrids, however, callus production was similar in rachides (67.7%) and peduncles (61.6%), and explants of young inflorescences yielded the highest amount of callus; callus tissue from the young inflorescences also showed the highest plant regeneration ability (Wojciechowska, 1992).

In our study, differentiation of plants from callus tissue depended on the genotype. The *T. aestivum* × *S. cereale* (ABDR) hybrid gave significantly higher regeneration from peduncle callus, but rachis callus from the *T. aestivum* × *T. monococcum* (AA^mBD) hybrid proved to be more efficient regeneration material. Generally, the quantitative parameters of plant regeneration differed significantly between these two hybrids. Callus of the *T. aestivum* × *S. cereale* (ABDR) hybrid showed low regeneration ability, and callus of *T. aestivum* × *T. monococcum* (AA^mBD) the highest. The tested genotypes differed in the presence or absence of the A^m and R genomes. Rye carries lower morphogenic potential than any wheat variety (Rybczyński, 1990; Bolesta, Rybczyński, 2001), so it might be concluded that the presence of the A^m genome stimulates regeneration, but that the presence of the R genome inhibits regeneration. Such a hypothesis would explain the observed intermediate regeneration ability of *T. tauschii* × amphiploid *T. monococcum*/*S. cereale* (A^mDR) hybrid, which includes both genomes. This suggestion requires further research on other genotypes differing in genotype constitution as our material did.

Production of plant regenerants from rachides and peduncles makes it possible to increase the amount of plant material of difficult-to-obtain hexaploid wheat hybrids with a directly introduced A^m genome from diploid wheat, a source of many new genes (Sodkiewicz et al., 2006). The introduction of the *T. tauschii* (D) genome into the A^mDR hybrid makes it possible to introduce other alleles and genes for practical applications (Sodkiewicz and Majewska, 2002). Our results should be helpful in developing protocols for multiplication of rare F_1 hybrids for research on amphiploidization, testing gene expression, and backcrossing trials.

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