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Doubled Haploid Production from Spanish and Central European Spelt by Anther Culture

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ABSTRACT

In recent years, spelt (*Triticum aestivum* (L.) ssp. *Spelta*) has become an added-value alternative crop to modern wheat. Spanish spelt constitutes a unique separate gene pool from central European germplasm. The availability of spelt Doubled Haploid (DH) production protocol is a great advantage to speed up breeding programs. This is the first study evaluating the ability of DH plant production, by anther culture, of five Spanish spelt landraces and three F_5 lines derived from Spanish spelt x bread wheat crosses. Two central European commercial varieties were also included in the analysis. DH plants were obtained from all material with the exception of one F_5 line. The Spanish spelt landraces produced more embryos/100 anthers (73-166.3) than the two European varieties (8.6-22.2). The main bottleneck in the Spanish germplasm was the high number of albino plants regenerated, with percentage of green plants lower than 13% in three of the landraces. Nevertheless, up to 15.6 and 1.8 green plants/100 anthers were obtained from the Spanish and the central European germplasm, respectively. A great variation in the percentage of spontaneous chromosome doubling was obtained, with 4 lines producing around 80% and 2 lines less than 15%. The ovary genotype used for anther co-culture is a critical factor to increase the efficiency of the system. Bread wheat 'Caramba' ovaries increased almost 6 times the number of green plants as compared to spelt landrace 'BG-1987' ovaries. This study shows that DH plants can be produced efficiently from Spanish spelt to be used in breeding programs.

Keywords: Ancient species, Anther culture, Spanish germplasm, Spontaneous chromosome doubling, *Triticum aestivum* (L.) ssp. *Spelta*.

INTRODUCTION

Spelt (*Triticum aestivum* (L.) Thell. ssp. Spelta) is an ancient hulled hexaploid wheat currently grown in Europe on more than 100,000 ha (Longin and Würschum, 2014). In recent years, spelt has gained renewed interest in developed countries due to current consumer trends towards regional crops that provide nutrients for a healthy diet (Longin and Würschum, 2016). In this sense, spelt has higher protein, lipid, and mineral (Mg, Fe, Cu, Zn and P) content than bread wheat (*Triticum aestivum* (L) (Ruibal-Mendieta *et al*., 2005; Escarnot *et al*., 2012). Furthermore, spelt produces more in less favorable growing conditions, and has lower fertilizer, herbicide and pesticide requirements than bread wheat (Campbell, 1997). Hence, spelt is suitable for stress conditions and organic farming systems (Konvalina *et al*., 2014). It is, therefore, an added-value alternative crop to modern wheat for farmers, millers, and bakers.

The exploitation of landraces and ancient species is a key factor to drive genetic improvements in plant breeding. Spelt has become a valuable gene resource for modern

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wheat breeding as it hybridizes easily with common wheat. Genetic diversity studies in Spanish spelt landraces and central European and Middle Eastern accessions showed that Spanish spelt landraces constitute a compact and separate gene pool from central European material (Elía *et al*., 2004). Two new alleles detected in Spanish spelt for the *Glu-B1* locus, four alleles for *Glu-D1*, and one *waxy* allele have not been described previously (Caballero *et al*., 2001; Guzmán *et al*., 2010), and could contribute to distinct bread- and noodle-making quality. This gene pool may also provide new sources of adaptation to environments in southern Europe that are poor in resources. Therefore, the Spanish spelt collection is a valuable and unique genetic resource that can be used in European bread wheat and spelt breeding programs.

The spelt or spelt x bread wheat breeding programs based on Doubled Haploid (DH) plants are of great interest to shorten the time needed to develop new varieties. Intergeneric crosses with maize and anther culture are the two methods with the highest potential for DH production in wheat. The number of bread wheat varieties selected from DH lines obtained by anther or interspecific crosses is increasing every year (Tadesse *et al*., 2012). In spelt, only one study was reported using intergeneric crosses, obtaining an average of 6.1 haploid plants/100 flowers from 6 breeding lines (Escarnot *et al*., 2014). Spelt anther culture response has been evaluated in two previous studies using central European germplasm and protocols developed for bread wheat. An average of 21 embryo-like structures and 0.9 green plantlets/100 anthers were produced from ten lines (Schmid, 1990) and, recently, a high frequency of green plantlets/100 anthers (30.6) was obtained from the Hungarian genotype 'GK Fehér' (Lantos *et al*., 2017). Since Spanish spelt constitutes a separate gene pool from the central European germplasm, evaluation of anther culture response should be assayed before setting up DH breeding programs with these materials.

One of the critical steps to achieve highly efficient wheat DH plant production by anther culture is to use ovary pre-conditioned medium and/or ovary co-culture, as well as the ovary genotype used for the co-culture (Castillo *et al*., 2015). Spelt contains higher levels of Cu and Zn than bread wheat (Ruibal-Mendieta *et al*., 2005), and both micronutrients are known to induce anther culture response in barley (Wojnarowiez *et al*., 2002; Echavarri *et al*., 2008). Therefore, spelt ovaries could be good candidates for co-culture.

The aim of this study was to evaluate the ability to produce doubled haploids by anther culture from Spanish spelt germplasm, using the standard bread wheat protocol developed in our laboratory. Furthermore, the potential of spelt ovaries to induce anther culture response was assayed.

MATERIALS AND METHODS

Material and Growing Conditions of the Donor Plants

In this study, we used five Spanish spelt landraces from the CRF-INIA (National Plant Genetic Resources Center): 'BG-1952' (L1), 'BG-1953' (L2), 'BG-1954' (L3), 'BG-1967' (L4) and 'BG-1987' (L5), meeting the spelt quality characteristics (high protein content and high extensibility), with shorter growth cycles than spelt cultivars from other European countries (Elia, 2007); three F_5 lines from spelt x bread wheat crosses: BG-1969 x 'Ingenio' (F_5-1), BG-1969 x 'Innov' (F_5-2), BG-13840 x Innov (F_5-3); two modern, high-yielding commercial spelt varieties developed by the University of Hohenheim, 'Zollernspelz'(L6) and 'Divimar' (L7); and the bread wheat commercial variety 'Caramba'. Donor plants were grown as described by Soriano *et al*. (2007).

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Preparation of Ovary Pre-Conditioned Medium and Ovary Co-Culture (OVPCM)

Ovaries were excised from flowers that contained microspores at a late binucleate stage of development. Ovaries were cultured in 2 mL of MS3M [MS medium modified by Hu and Kasha (1997), containing 62 g L^{-1} maltose, 1 mg L^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D), 1 mg L^{-1} BenzylAdenine (BA)], supplemented with 200 g L^{-1} Ficoll Type 400 (Sigma) (MS3MF200) (Soriano *et al*., 2007). Cultures were kept in the dark at 25ºC for 5 days before anther culture. The ovaries (6 ovaries per 2 ml) were kept in MS·MF200 during anther culture (OVPCM).

Anther Culture

Anthers containing the majority of microspores at the mid- to late-uninucleate stage were pre-treated with 127.5 g L^{-1} mannitol (Sigma), 5.9 g L^{-1} $CaCl_2$ plus FHG macronutrients (Hunter, 1987) solidified with 0.8 g L^{-1} Sea Plaque Agarose (Lonza) (Soriano *et al*., 2007). After 5 days, swollen anthers were selected and treated with 0.2% n-butanol for 5 hours in MS3M liquid medium as described by Soriano *et al*. (2008). After n-butanol treatment, anthers were cultured in 2 mL of OVPCM containing 7 ovaries in 3 cm Ø Petri dishes. After 10 to 12 days, plates were replenished with 2 mL of the MS3MF400 (MS3M supplemented with 400 g L^{-1} Ficoll Type 400). Cultures were kept in the dark at 25ºC.

Experiments

Experiment 1: DH Production from Spanish and Central European Spelt by Anther Culture

Anthers from five Spanish spelt landraces, three F_5 lines from spelt x bread wheat crosses, and two central European commercial varieties were pre-treated and cultured as described above. Ovaries from bread wheat cultivar Caramba were used for OVPCM.

Experiment 2: Potential of Spelt Ovaries to Induce Spelt Anther Culture Response

Anther culture response from spelt landraces "BG-1953" and "BG-1967" was evaluated in OVPCM medium, using ovaries from the spelt landrace BG-1987 or the bread wheat cultivar Caramba (treatments).

Plant Regeneration, Soil Transfer, Ploidy Analysis and Colchicine Treatment

Embryos developed after 30 days were transferred to 6 cm Ø Petri dishes containing J25-8 medium (Jensen, 1977) for regeneration. Embryos were kept in the dark at 25ºC for 2 days and then transferred to the light. After 20 days, plants were transferred to Magenta boxes containing the same medium plus 2 mg L^{-1} NAA for root development. Soil transfer and ploidy analysis were performed as described by Soriano *et al*. (2007). DH plants were transferred to greenhouse for seed production. For colchicine treatment, haploids plants with 2-3 tillers were removed from the soil and roots were washed and cut back to about 2 cm below the crown. Plants were then immersed to a depth of 5 cm in a 0.1% aqueous colchicine (Sigma D-4540) solution containing 2% DMSO for 5 hours at 24°C (modified from Devaux, 2003). Plants were rinsed in running tap water for 15 minutes, potted in soil and transferred to the greenhouse at 24°C for seed production.

Statistical Analysis

Twelve to 23 replicates of 35 anthers per genotype were used in each experiment.

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Within replicates, pre-treated anthers from the same spike were randomly distributed between treatments in Experiment 2. The numbers of embryos, green, and albino plantlets and the number of DH plantlets/100 anthers were recorded, then, regeneration percentages (number of regenerated plantlets/100 embryos), green plantlets (number of green plantlets/100 total plantlets), and spontaneous chromosome doubling (number of DH/100 analyzed plantlets) were calculated. Experiments were established in a completely randomized design. Statistical analysis was performed with SAS software (SAS Institute Inc., Cary, NC, and Version 9.4). Normality and homogeneity of variance were tested using Kolgomorov-Smirnoff and Levene´s tests, respectively. The variables number of embryos and albino plants were transformed using the square root (X+0.5), and number of green plants were transformed using log (x+1) to meet parametric assumptions. Percentages of plant regeneration and green plants did not need transformation. The GLM (Generalized Linear Model) procedure was used to conduct the ANOVA for all variables, except for percentage of spontaneous chromosome doubling, which was analyzed using the FREQ procedure to do the Chi Square test. The Duncan method ($\alpha \leq 0.05$) was used for the mean separation.

RESULTS

Experiment 1: DH Production from Spanish and Central European Spelt by Anther Culture

Evaluation of anther culture response was performed following the protocol developed in our laboratory for bread wheat. ANOVA showed that the number of embryos, green, and albino plants and the percentages of plant regeneration and green plants were strongly affected by the genotype (Table 1). Although a wide variation was observed between materials, embryos were obtained from all lines (Figure 1). Spanish material produced the most embryos, especially landraces BG-1987 and BG-1954, with, respectively, 166 and 120 embryos/100 anthers (Figures 1 and 3-c), and the other 3 landraces and F_5-3 line with 73-84 embryos (Figures 1 and 3-a). Significantly fewer embryos were obtained from the other F_5 lines (17-27) and the central European varieties Zollernspelz and Divimar (8-22) [Figures 1 and 3 (e and g].

Green plantlets were also obtained from all the lines except the F_5-1 (Figure 1). Among the Spanish landraces, BG-1954 (Figure 3-d) and BG-1967 produced the highest number of green plantlets/100 anthers with 16 and 9, respectively, and BG-1987 the lowest number with 2.8. A

Table 1. ANOVA of Experiments 1 and 2 for the variables numbers of embryos (NEmb), green plants (NGP), albino plants (NAP) and percentages of regeneration (Reg) and green plants (GP). The variables NEMB and NAP were transformed with square root (X+0.5) and NGP with log (X+1).

	Source	*df*	Mean squares				
			NEmb[a]	NGP[a]	NAP[a]	Reg (%)	GP (%)
Exp 1	Genotype	9	159.37***	5.94***	39.00***	975.23*	4595.91***
	Error	121	11.62	1.07	2.77	458.50	497.24
EXp 2	Anther genotype (AG)	1	106.80*	0.08 ns	71.88***	1074.20**	334.47 *
	Ovary genotype (OG)	1	72.64*	13.25**	0.55 ns	668.96**	1400.84***
	AG X AO	1	17.05 ns	0.29 ns	6.24 ns	0.99 ns	511.39*
	Error	48	15.65	0.85	3.69	61.62	74.35

[a] values based on 100 anthers; ns, *, **, *** Not significant, significant at 0.05, 0.01 and 0.001 levels of probability, respectively

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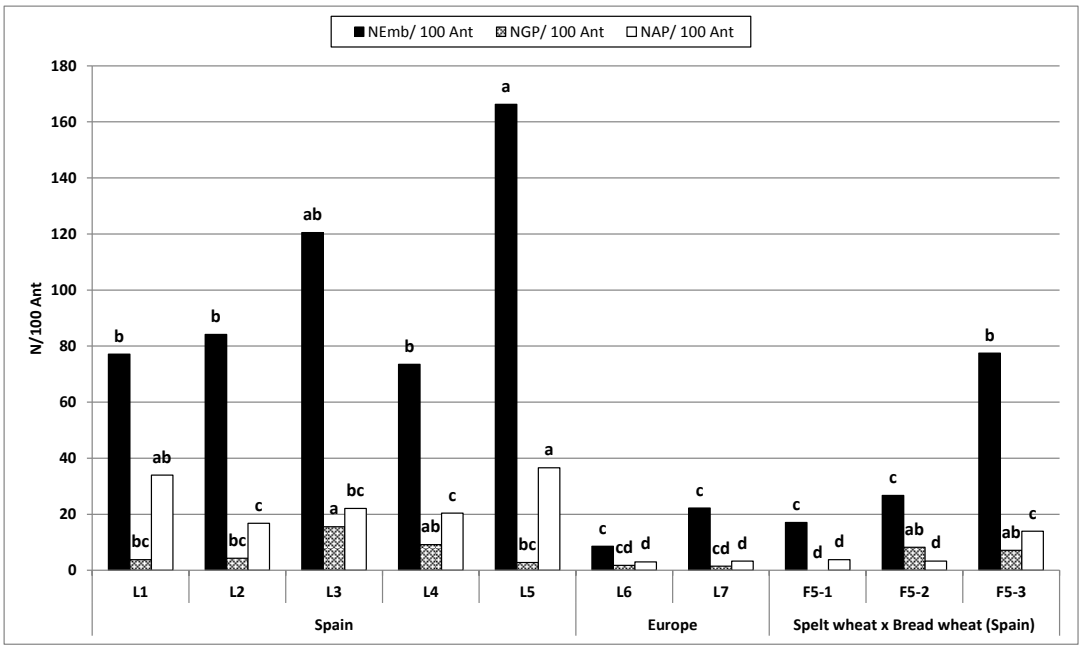

Figure 1. Number of embryos and green plants obtained per 100 anthers from 5 Spanish spelt wheat landraces BG-1952, BG-1953, BG-1954, BG-1967 and BG-1987 (L1-L5, respectively), the central European commercial spelt cultivars Zollernspelz and Divimar (L6 and L7, respectively) and 3 F_5 lines from spelt wheat x bread wheat crosses (BG-1969×Ingenio, BG-1969×Innov, and BG-13840×Innov, F_5-1 to F_5-3, respectively).

low number of green plants was obtained from both central European varieties (1.5-1-8). In all the materials, there were more albino plants than green plants, excluding F_5-2 (Figures 1 and 3-h), which had 62% of green plants (Figure 2). BG-1954 and F_5-3 percentages of green plantlets were similar to the two European varieties (31-47%) [Figures 2 and 3 (d and f)]. Three out of the 5 landraces showed less than 13% of green plantlets (Figures 2 and 3-b).

The two European varieties produced the lowest percentage of plantlet regeneration (16-20%) (Figures 2 and 3-f). Higher rates were obtained from Spanish material: BG-1953, BG-1954, BG-1987, F_5-1 and F_5-3 showed a plantlet regeneration of 26-33% (Figures 2 and 3-d), and BG-1952, BG-1967 and F_5-2 showed an even higher plantlet regeneration of 43-46% [Figures 2 and 3 (b and h)].

Statistically significant differences were also observed between materials for spontaneous chromosome doubling (Figure 2). Landrace BG-1952, the two European varieties, and the F_5-2 line showed approximately 80% spontaneous doubling. The lowest percentages were obtained from BG-1967 and F_5-3 with less than 15%. Haploid plants from BG-1952, BG-1954, BG-1967, and BG-13840 x Innov were treated with colchicine (Table 2). Rates of duplication higher than 75% were obtained in 3 out of the 4 lines and 40 % mortality in BG-1967. An average of 55-126 seed/plant was harvested from colchicine treated plants. DH plants from all lines and colchicine-treated haploids plants were transferred to the greenhouse for seed production (Figure 4-a). F_{5-2} and F_{5-3} DH plants were given to breeders for agronomical characterization in the field (Figure 4-b).

Experiment 2: Potential of Spelt ovaries to Induce Spelt Anther Culture Response

A great anther and ovary genotypic effect was shown by ANOVA (Table 1). Anther genotype strongly affected all the variables studied with the exception of number of green plants and percentage of spontaneous doubling (Tables 1 and 3). Statistically significant differences were also observed

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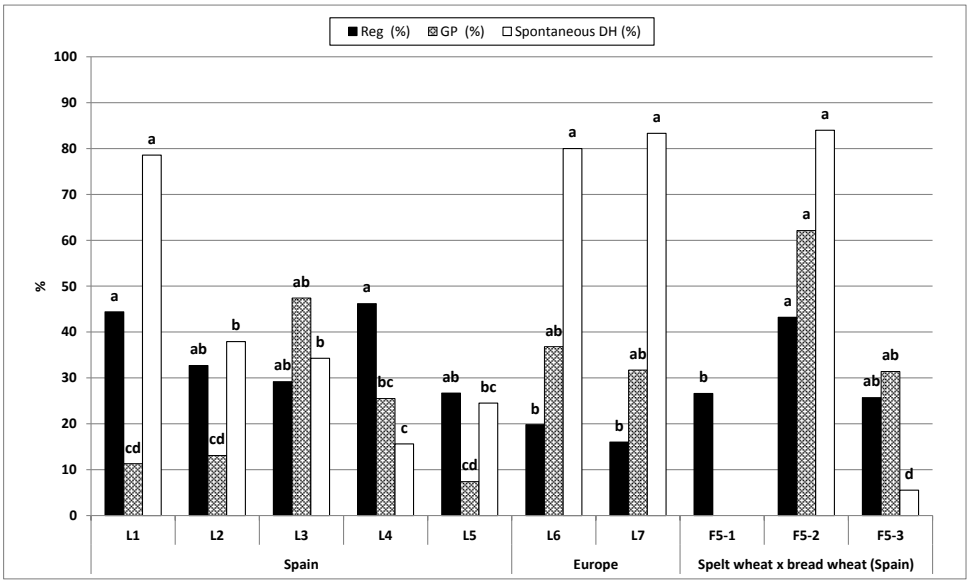

Figure 2. Percentages of plant regeneration (Reg), green plants (GP) and spontaneous doubling from 5 Spanish spelt wheat landraces BG-1952, BG-1953, BG-1954, BG-1967 and BG-1987 (L1-L5, respectively), the European spelt cultivars Zollernspelz and Divimar (L6 and L7, respectively) and 3 F_5 lines from spelt wheat x bread wheat (BG1969×Ingenio, BG-1969×Innov, and BG-13840×Innov, F_5-1 to F_5-3, respectively).

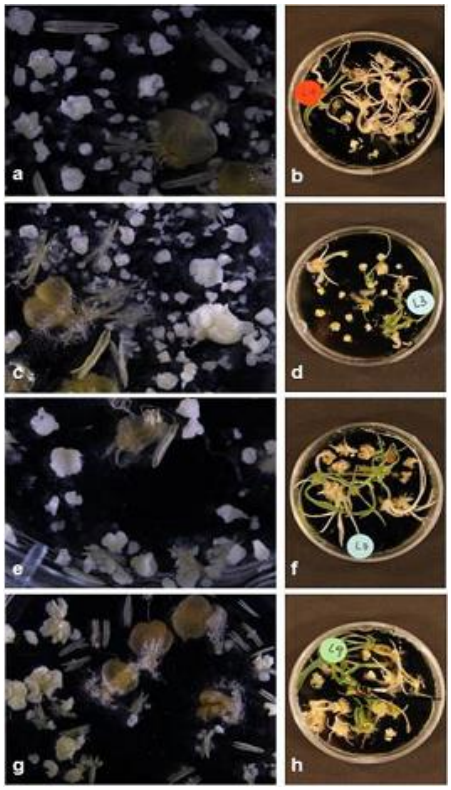

Figure 3. Microspore embryogenesis and plant regeneration from BG-1952 (L1) (a, b), BG-1954 (L3) (c, d), the European spelt cultivar Divimar (L7) (e, f), and F_5 line from BG-1969×Innov (F5-2) (g, h). (a, c, e, g= 10X magnification; b, d, f, h= 6 cm Ø Petri dishes).

between ovary genotypes for all the variables, excluding the number of albino plants. Caramba ovaries produced almost a 6-fold increase in the number of green plants and percentage of green plants, as compared to BG-1987 (Table 3). Interaction of anther x ovary genotype was observed for percentage of green plants (Table 1). A 14- and a 3-fold increase in the value of this variable was obtained in BG-1953 and BG-1967 anther culture, respectively, with Caramba ovaries (data not shown).

DISCUSSION

In this study, the ability of DH plant production from five Spanish spelt landraces and three F_5 lines from Spanish spelt x bread wheat crosses was evaluated for the first time using the protocol developed in our laboratory for bread wheat. The Spanish landraces produced a higher average number of embryos/100 anthers (104) than the two central European commercial varieties included in this study (15). A low number of embryos was also reported with central

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Figure 4. Spelt doubled haploid plants growing in the greenhouse for seed production (a) and agronomic evaluation of DH plants from BG-1969×Innov (F_5-2) and BG-13840×Innov (F_5-3) (b) (arrows, dark green plants).

Table 2. Percentages of plant duplication and mortality, and number of seeds after colchicine treatment in different lines of spelt wheat.

Line	Colchicine Treatment			N Seeds/ Duplicated plant
	N Plants	Duplication (%)	Mortality (%)	
BG-1952	3	100	0	55
BG-1954	16	75	12	79
BG-1967	25	40	60	68
BG-13840 x Innov	17	94	6	126

Table 3. Effect of the anther and ovary genotype on spelt wheat anther response. .

Genotype		NEmb**/ 100 Ant	NGP/ 100 Ant	NAP/ 100 Ant	Reg (%)	GP (%)	Spontaneous DH (%)
Anthers	BG-1953	130.1 b	3.5 a	22.6 b	18.9 b	9.0 a	40.0 a
	BG-1987	203.5 a	2.2 a	48.8 a	27.9 a	3.9 a	25.0 a
Ovary	Caramba	195.2 a*	5.1 a	34.6 a	19.5 b	12.1 a	37.1 a
	BG-1987	140.0 b	0.9 b	36.3 a	26.8 a	1.6 b	25.0 a

** NEmb = number of embryos; NGP = number of green plants; NAP = number of albino plants; Reg % = percentage of plant regeneration; GP % = percentage of green plants; * Values followed by the same letter within anther genotype and ovary genotype are not significantly different (P<0.05)

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European material used in previous studies (Schmid, 1990; Lantos *et al*., 2017).

A low embryo-plantlet conversion rate has been described to be a major bottleneck in the anther culture of central European spelt (Schmidt, 1990), which is normally associated with small and/or non-differentiated embryos (Kim *et al*., 2013). Our results with the two European varieties are in accordance with previous studies, producing the lowest percentage of plantlet regeneration (16-20%). However, three Spanish lines showed more than 40% plant regeneration.

The regeneration of chlorphyll-deficient (albino) plants did not seem to be a major problem in previous studies of spelt anther culture, since 80-90% of regenerated plants were green (Schmidt 1990; Lantos *et al*., 2017). However, albinism seems to be the main bottleneck in Spanish spelt, with 3 of the landraces showing less than 13% of green plantlets. These results could indicate that Spanish spelt germplasm could have alleles that favor the production of albino plants in anther culture. In wheat anther culture, albinism is a major hurdle as compared to intergeneric crosses, where almost no albino plants are produced (Wedzony *et al*., 2009). Genetic and physiological factors are known to influence the frequency of albino plants (Dwivedi *et al*., 2015).

In this study, green plantlets were obtained from all the lines, except the F_5-1, despite the low percentages of plantlet regeneration and green plants from most of the material. The number of green plantlets/100 anthers obtained from the 5 Spanish landraces (3-16) is quite similar to those described previously with different bread wheat genotypes (0.04-28.7 green plant/100 anthers) by Lantos *et al*. (2013). In the central European varieties, the small number and low quality of embryos could condition the lowest number of green plants (1.5-1-8). These results are in accordance with those reported in 10 central European spelt lines (Schmidt, 1990).

Our results suggested that, in general, the Spanish spelt germplasm could respond better than the central European germplasm despite its high percentage of albinism. Differences in anther culture response according to the origin of the material (Eastern vs. North Western Europe) have been described previously in bread wheat (Holme *et al*., 1999). Further studies with a larger number of Spanish landraces would be desirable to confirm these results, since certain genotypes such as GK Fehér produced a higher number of green plants/100 anthers (30.6) than the best Spanish landrace (Lantos *et al*., 2017).

Identification of materials with a high androgenetic response is important when DH technology is being used in crop improvement. In this sense, landraces BG-1954 and BG-1967, that rendered the highest number of green plants, showed similar values for almost all variables. In addition, a short genetic distance between both landraces was reported in a genetic analysis performed on a collection of 100 Spanish spelt landraces (Elia, 2007). Therefore, it is possible to suggest that these lines had similar alleles for these traits. Also, Innov bread wheat cultivar could have alleles that favor the percentage of green plants since F_5-2 (BG-1969 x Innov) and F_5-1 (BG1969 x Ingenio) produced the highest and lowest percentage of green plants (62% and 0%, respectively).

The obtaining of a high percentage of spontaneous chromosome doubling in anther or microspore culture is a clear advantage over interspecific crosses or gynogenesis, where 10-15% rates have been reported (Maluszynski *et al*., 2003; Bohanec, 2009). In bread wheat anther culture, rates of 25-70% have been described (Maluszynski *et al*., 2003). In this study, a great variation in the percentage of spontaneous doubling (from 6-84%) was obtained depending on the material. The central European varieties showed percentages (around 80%) similar to that reported from GK Fehér (Lantos *et al*., 2017). However, this percentage was significantly higher than 30% reported in ten

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central European spelt lines (Schmid, 1990) and 40% of the five Spanish landraces. Other factors besides the genotype have been reported to influence the percentage of spontaneous doubling such as the stage of microspore development and the type of stress treatment (Castillo *et al*., 2009).

Spelt lines also showed different responses to colchicine treatment. Plants from 3 out of the 4 lines treated with colchicine showed high rates of survival and seed set (over 75%). These results agreed with those described in bread wheat (Castillo *et al*., 2009). The genotype, species, and the health and vigor of the plant material has been reported to influence the rates of induced duplication in cereals (for review see Castillo *et al*., 2009).

In bread wheat anther culture, ovary co-culture has been proved to increase the efficiency of DH production. Furthermore, the ovary genotype used is a critical factor to increase embryogenesis and spontaneous doubling (Castillo *et al*., 2015). Spelt ovaries were expected to enhance DH production due to their high Zn and Cu content (Ruibal-Mendieta *et al*., 2005), which are known to increase the efficiency of barley anther culture (Wojnarowiez *et al*., 2002; Echavarri *et al*., 2008). However, bread wheat Caramba ovaries produced almost a 6-fold increase in the number of green plants/100 anthers as compared to spelt BG-1987 ovaries. These results agree with those reported by Castillo *et al*. (2015), showing that Caramba and Tigre ovaries were the most efficient in comparison with other bread wheat cultivars. More spelt genotypes should be screened in order to confirm the low promoting effect of spelt ovaries on anther culture response.

This study demonstrated that DH plants from Spanish and central European spelt germplasm can be efficiently produced with a bread wheat anther culture protocol developed in our laboratory, using bread wheat ovaries for co-culture. Spanish germplasm produced higher numbers of embryos and green plants and higher percentages of plant regeneration than the two central European commercial varieties evaluated. The number of green plants obtained ensures the viability of Spanish spelt or spelt x bread wheat DH-based breeding programs.

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REFERENCES

1. Bohanec, B. 2009. Doubled Haploids *via* Gynogenesis. In: "*Advances in Haploid Production in Higher Plants"*, (Eds): Touraev, A., Forster, B. P. and Jain, S. M. Springer Science+Business Media BV, Netherlands, PP. 35-46.
2. Caballero, L., Martín, L. M. and Alvarez, J. B. 2001. Allelic Variation of the HMW Glutenin Subunits in Spanish Accessions of Spelt Wheat (*Triticum aestivum* ssp. *spelta* L. em Thell). *Theor. Appl. Genet.*, **103:** 124-128.
3. Campbell, K. G. 1997. Spelt: Agronomy, Genetics and Breeding. *Plant Breed. Rew.*, **15:** 187-213.
4. Castillo, A. M., Cistué, L., Vallés, M. P. and Soriano, M. 2009. Chromosome Doubling in Monocots. In: "*Advances in Haploid Production in Higher Plants"*, (Eds): Touraev, A., Forster, B. P. and Jain, S. M. Springer Science+Business Media BV, Netherlands, PP. 329-338.
5. Castillo, A. M., Sánchez-Díaz, R. A. and Vallés, M. P. 2015. Effect of Ovary Induction on Bread Wheat Anther Culture: Ovary Genotype and Developmental Stage, and Candidate Gene Association. *Front. Plant Sci.*, **6:** 402.
6. Devaux, P. 2003. The *Hordeum bulbosum* (L.) Method. In: "*Doubled Haploid Production in Crop Plants"*, (Eds.): Maluszynski, M., Kasha, K. J., Forster, B. P. and Szarejko, I. Kluwer Academic Publishers, Dordrecht, PP. 15-19.

[DOR: 20.1001.1.16807073.2019.21.5.11.8]

7. Dwivedi, S. L., Britt, A. B., Tripathi, L., Sharma, S., Upadhyaya, H. D. and Ortiz, R. 2015. Haploids: Constraints and Opportunities in Plant Breeding. *Biotech. Adv.*, **33:** 812-829.
8. Echavarri, B., Soriano, M., Cistué, L., Valles, M. P. and Castillo, A. M. 2008. Zinc Sulfate Improved Microspore Embryogenesis in Barley. *Plant Cell Tiss. Organ. Cult.*, 93: 295–301.
9. Elia, M. 2007. Estudios Previos a la Mejora Genética de la Escanda (*Triticum spelta* L.): Uso de la Colección Española. Doctoral Thesis, Univ Lleida, Lleida, Spain.
10. Elia, M., Moralejo, M., Rodriguez-Quijano, M. and Molina-Cano, J. L. 2004. Spanish Spelt. A Separate Gene Pool within the Spelt Germplasm. *Plant Breed.*, **123:** 297-299.
11. Escarnot, E., Jacquemin, J. M., Agneessens, R. and Paquot, M. 2012. Comparative Study of the Content and Profiles of Macronutrients in Spelt and Wheat: A Review. *Biotechnol. Agron. Soc. Environ.*, **16(2):** 243-256.
12. Escarnot, E., Tibaut, C. and Forgeois, P. 2014. Study of the Impact of Growth Substance Treatment and Maize (*Zea mays* L.) Variety in Spelt (*Triticum spelta* L.) Haplodiploidization. *Biotechnol. Agron. Soc. Environ.*, **18(1):** 32-36.
13. Guzmán, C., Caballero, L., Moral, A. and Alvarez, J. B. 2010. Genetic Variation for Waxy Proteins and Amylose Content in Spanish Spelt Wheat (*Triticum spelta* L). *Genet. Resour. Crop Evol.*, **57**: 721-725.
14. Holme, I. B., Olesen, A. and Andersen, S. B. 1999. Anther and Isolated Microspore Culture Response of Wheat Lines from Northwestern and Eastern Europe. *Plant Breed.*, **118:** 111–117.
15. Hu, H. and Kasha, K. J. 1997. Improvement of Isolated Microspore Culture of Wheat (*Triticum aestivum* L.) through Ovary Co-Culture. *Plant Cell Rep.*, **16:** 520-525.
16. Hunter, C. P. 1987. *European Patent Application Nr0245898 A2*. PP. 1-8
17. Jensen, C. J. 1977. Monoploid Production by Chromosome Elimination. In: "*Applied and Fundamental Aspects of Plan Cell, Tissue and Organ Culture",* (Eds.): Reinert, J. and Bajaj, Y. P. S. Springer, Berlin, PP. 299-330.
18. Kim, M., Park, E. -J., An, D. and Lee, Y. 2013. High-Quality Embryo Production and Plant Regeneration Using a Two-Step Culture System in Isolated Microspore Cultures of Hot Pepper (*Capsicum annuum* L.). *Plant Cell Tiss. Organ. Cul.*, **112:** 191-201.
19. Konvalina, P., Stehno, Z., Capouchová, I., Zechnaer, E., Berger, S., Grausgruber, H., Janovská, D. and Moudry, J. 2014. Differences in Grain/Straw Ratio, Protein Content and Yield in Landraces and Modern Varieties of Different Wheat Species under Organic Farming. *Euphytica*, **199(1-2):** 31-40.
20. Lantos, C., Weyen, J., Orsini, J.M., Gnad, H., Schlieter, B., Lein, V., Kontowski, S., Jacobi, A., MihÄly, R., Broughton, S. and Pauk, J. 2013. Efficient Application of *In Vitro* Anther Culture for Different European Winter Wheat (*Triticum aestivum* L.) Breeding Programmes. *Plant Breed.*, **132:** 149–154.
21. Lantos, C., Jenes, B., Bóna, L., Cserháti, M. and Pauk, J. 2017. High Frequency of Doubled Haploid Plant Production in Spelt Wheat. *Acta Biologica Cracoviensia S Botanic*a, **58(2):** 107-112.
22. Longin, C.F.H. and Würschum, T. 2014. Genetic Variability and Correlation among Agronomic and Disease Resistance Traits in a Diversity Panel and Elite Breeding Material of Spelt Wheat. *Plant Breed*., **133:** 459-464.
23. Longin, C. F. H. and Würschum, T. 2016. Back to the Future: Tapping into Ancient Grains for Food Diversity. *Trends Plant Sci*., **21(9):** 731-737.
24. Maluszynski, M., Kasha, K.J., Foster, B. P. and Szarejko, I. 2003. *Doubled Haploid Production in Crop Plants: A Manual*. Kluwer Academic Publishers, Dordrecht, PP.1-428.
25. Ruibal-Mendieta, N. L., Delacroix, D., Mignolet, E., Pycke, J. M., Marques, C., Rozenberg, R., Petitjean, G., Habib-Jiwan, J. L., Meurens, M., Quetin-Leclercq, J., Delzenne, N. M. and Larondelle, Y. 2005. Spelt (*Triticum aestivum* ssp Spelta) as a Source of Breadmaking Flours and Bran Naturally Enriched in Oleic Acid and Minerals but Not Phytic Acid. *J. Agric. Food Chem.*, **53:** 2751-2759.
26. Schmid, J. 1990. *In Vitro* Production of Haploids in *Triticum spelta*. In: "*Biotechnology in Agriculture and Forestry-13 Wheat"*, (Ed.): Bajaj, Y. P. S. Springer-Verlag, Berlin, Heildelberg, PP. 363-381.
27. Soriano, M., Cistué, L. and Castillo, A. M. 2008. Enhanced Induction of Microspore Embryogenesis after n-Butanol Treatment in Wheat (*Triticum aestivum* L.) Anther Culture. *Plant Cell Rep.*, **27:** 805-811.
28. Soriano, M., Cistué, L., Vallés, M. P. and Castillo, A. M. 2007. Effects of Colchicine on Anther and Microspore Culture of Bread

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Wheat (*Triticum aestivum* L.). *Plant Cell Tiss. Organ. Cul.*, **91:** 225-234.
29. Tadesse, W., Inagaki, M., Tawkaz, S., Baum M. and van Ginkel, M. 2012. Recent Advances and Application of Doubled Haploids in Wheat Breeding. *Afr. J. Biotech.*, **11(89):** 15484-15492.
30. Wedzony, M., Forster, B. P., Zur, I., Golemiec, E., Szechynska-Hebda, M., Dubas, E. and Gotebiowska, G. 2009. Progress in Doubled Haploid Technology in Higher Plants. In: "*Advances in Haploid Production in Higher Plants"*, (Eds): Touraev, A., Forster, B. P. and Jain, S. M. Springer Science+Business Media BV, Netherlands, PP. 1-34.
31. Wojnarowiez, G., Jacquard, C., Devaux, P., Sangwan, R. S. and Clément, C. 2002. Influence of Copper Sulphate on Anther Culture in Barley (*Hordeum vulgare* L). *Plant Sci.*, **162:** 843-847.

تولید هاپلوئید مضاعف از گندم پوشان (spelt) اسپانیایی و اروپای مرکزی با کشت بساک

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چکیده

در سال های اخیر، گندم پوشان (spelt) با نا م علمی (*Triticum aestivum* (L.) ssp. *Spelta*) برای گندم مدرن گزینه ای با ارزش افزوده شده است. گندم پوشان اسپانیایی دارای خزانه ژنی منحصر به فرد و مجزا از ژرم پلاسم های اروپای مرکزی است. برای سرعت دادن به برنامه های اصلاح نژاد، دردسترس بودن دستورالعمل تولید هاپلوئید مضاعف گندم پوشان (DH) بسیار مفید است. این نخستین پژوهشی است که توانایی تولید گیاه DH با روش کاشت بساک از ۵ رقم بومی (landrace) و ۳ مورد لاین F_5 مشتق از تلاقی گندم پوشان اسپانیایی x گندم نان را ارزیابی میکند. این تحلیل دو رقم تجاری اروپای مرکزی را نیز شامل بود. از همه مواد (گیاهی) مطالعه شده، به استثناء یک لاین F_5 ، گیاهان DH به دست آمد. به ازای ۱۰۰ بساک، در رقم های بومی گندم پوشان اسپانیایی تعداد جنین تولیدی (۱۶۶.۳–۷۳) در مقایسه با دو رقم اروپای مرکزی (۲۲/۲–۸/۶) بیشتر بود. گلوگاه اصلی در ژرم پلاسم اسپانیایی زیاد بودن تعداد گیاهان زال (albino) باززایی شده بود در حالیکه که درصد گیاهان سبز در سه رقم بومی کمتر از ۱۳٪ بود. با این وجود، از ژرم پلاسم های اسپانیایی و اروپای مرکزی، به ترتیب تا حد ۱۵/۶ و ۱/۸ گیاه سبز به ازای ۱۰۰ بساک به دست آمد. درصد دو برابر شدن خود به خودی کروموزوم ها (spontaneous chromosome doubling) تغییرات زیادی نشان داد، به طوری که ۴ لاین ۸۰٪ و ۲ لاین کمتر از ۱۵٪ داشتند. در روش همراه–کشتی (co-culture) بساک، ژنوتیپ جنین مورد استفاده عامل مهمی در افزایش سامانه است. جنین گندم نان 'Caramba' تعداد گیاهان سبز را تا ۶ برابر جنین رقم بومی گندم پوشان 'BG-1987' افزایش داد.

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این پژوهش نشان می دهد که برای استفاده در برنامه های اصلاح نژاد، می توان گیاهان DH را از گندم پوشان اسپانیایی با کارآیی بالا تولید کرد.

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