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HAPLOID PRODUCTION VARIATION IN SEVERAL DURUM WHEAT CULTIVARS AND THEIR SYNTHETIC HEXAPLOID DERIVATIVES

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Abstract

Bread wheat haploids are being routinely produced by the sexual wheat/maize crossing protocol with perfect outputs across all its genotypes. A similar success however, has not been achieved for durum wheat cultivars. We evaluated 44 elite durum (*Triticum turgidum* L; 2n = 4x = 28, AABB) high yielding, widely adapted cultivars for their ability to generate haploids (n = 2x = 14, AB) when crossed by *Zea mays* (2n = 2x = 20). This gave a range from 0% to 12.3% across the 44 genotypes. Synthetic hexaploid (SH's) wheats derived from each of the 44 cultivars x *Aegilops tauschii* accessions gave a haploid frequency range from 2.5 to 42.6% providing unequivocal evidence that the D genome of *Ae. tauschii* significantly influenced haploid production efficiency in SH wheats. Durum cultivars amenable to haploidy induction are good candidates for molecular studies and gene transfer programs where doubled haploids have a distinct advantage.

Introduction

Haploid wheat plants (polyhaploids; n = 3x = 21, ABD) form the initial step from which homozygous progeny results via colchicine induced doubling. In earlier use have been techniques associated with anther culture or crosses of bread wheat by *Hordeum bulbosum* (Barclay, 1975). Both procedures have limitations (Picard, 1989; Snape *et al*., 1979) that prevent their application in wheat breeding programs where a wide spectrum of genotypic diversity exists. Crosses of bread wheat by maize (*Zea mays*) as a source for obtaining polyhaploids across all bread wheat genotypes has recently emerged as an effective procedure after the pioneering work of Laurie & Bennet (1986, 1987) in which the hormonal influence of 2,4 di-chloro–phenoxy acetic acid plays a critical role (Suenaga & Nakajima, 1989). Pearl millet and *Tripsacum* pollen sources also serve an identical role in haploid production as maize (Ahmad & Comeau, 1990; Riera-Lizarazu & Mujeeb-Kazi, 1993).

Bread wheat haploids are now being produced very routinely and simplistically (Mujeeb-Kazi *et al*., 2002). Their use is in wheat cytogenetics, wide crosses, wheat breeding, genetic analyses with extensions of the application into genetic engineering and molecular mapping (Mujeeb-Kazi, 2000). Mean frequency percentages ranges for embryo recovery, plantlet differentiation, and chromosome doubling over long-term experimentations (Mujeeb-Kazi, 2000) involving various bread wheat cultivars/maize crosses are 20 to 25, 80 to 90 and 80 to 95% respectively. Since then, significant improvements in regeneration and doubling have emerged giving 100% outputs for each (Mujeeb-Kazi *et al*., 2002). In essence haploid production for bread wheat is 100% effective across all habits (spring, winter, facultative) and genotypes.

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A similar success however, has not been realized for durum wheats where genotypic specificity is prevalent (Riera-Lizarazu *et al*., 1992; Amrani *et al*., 1993; Sarrafi *et al*., 1994; O'Donoughue & Bennett, 1994a, b; Almouslem *et al*., 1998; Inagaki & Hash, 1998). The absence of the D genome in durum wheats is reportedly a factor (Almouslem *et al*., 1998; Inagaki *et al*., 1997, 1998a) apart from the AABB durum genomic variation. In this paper we present our findings that demonstrate durum diversity for haploid production across 44 cultivars. The study further elucidates the contribution of the D genome of *Aegilops tauschii* to the 44 durum cultivars as measured by haploid production of their 44 derived synthetic hexaploid wheats (*T. turgidum/Ae. tauschii* = synthetic hexaploid; 2n = 6x = 42, AABBDD).

Materials and Methods

Plant materials: Field grown plants of 44 durum cultivars (*T. turgidum*; 2n = 4x = 28, AABB) and each of their derived synthetic hexaploids (*T. turgidum* x *Ae. tauschii*; 2n = 6x = 42, AABBDD) grown at El Batan, CIMMYT, Mexico, were used for haploid production. A single cross maize hybrid CML-246 x CML-242 was used as the pollen source. These plants were grown in potted soil and kept in the greenhouse conditions maintained at 26ºC day/14ºC night temperatures with 14 hours of natural light and approximately 65% relative humidity. Three plantings at 10-day intervals were made to ensure a continuous supply of fresh pollen.

Crossing procedures, detached tiller culture and embryo rescue: Spikes on detached tillers of all durum cultivars and synthetic hexaploids (SH) were kept in water, then hand-emasculated and pollinated after two days with fresh maize pollen. The haploid production protocols were similar to those reported by Mujeeb-Kazi *et al*., (2002). The pollinated spikes were cultured in a nutrient solution containing 40g/l sucrose, 100 mg/l 2,4-di-chloro-phenoxy acetic acid, 8 ml/l sulfurous acid and kept in a growth chamber. To facilitate embryo formation and development, the growth chamber conditions were maintained at continuous 22.5ºC temperature, 12 hour day length, and 60 to 70% relative humidity. After 14 to 16 days of pollination the seed set were removed from each spike, counted, sterilized and dissected under a stereomicroscope to excise the formed embryos. Subsequently, embryo formation frequencies were calculated for each durum genotype and its related SH wheat derivative. There were about 5 to 7 spikes tested for each entry. The embryo rescue, cold treatment, regeneration and transplantation procedures were similar to those of Mujeeb-Kazi *et al*., (1987). Cytology protocols of Mujeeb-Kazi *et al*., (1994) allowed validation of the haploid status i.e. n = 3x = 21, (ABD) or n = 2x = 14, (AB), and also of their meiotic associations on separated test plants for a few haploids. After three weeks of growth all haploid plants were treated with colchicine as a root-treatment procedure (Mujeeb-Kazi & Riera-Lizarazu, 1996). Successful chromosome doubling was inferred from seed setting on the colchicine-treated polyhaploid plants.

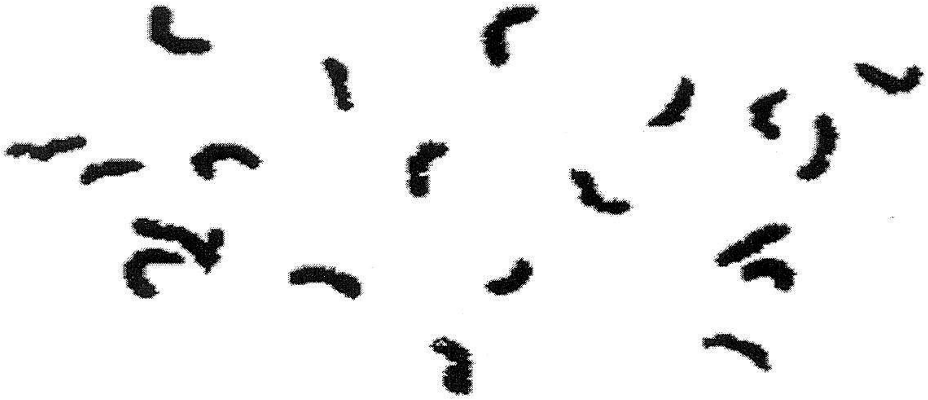
Results and Discussion

Initiation to embryo excision and regenerative cytology: The detached tillers initially kept in containers with tap water were emasculated after 1 day and covered with a plastic bag to enhance humidity. After pollination the water was replaced with the nutrient mix and 2,4-D (4 days) and allowed to grow for 15 days when seed set on the spikes became very pronounced. The perfect seed set on the spikes is more a function of the hormone 2,4-D that being systemic, influences the maternal tissues to become turgid. After 15 days post-pollination all set seed were extracted from the spikes, sterilized and subjected to embryo excision. Not all seed possessed embryos. If an embryo was present then it was minute, translucent, without scutellum appearance and was floating in the endosperm cavity filled with a watery solution. The watery endosperm is a good indicator of a haploid event. The embryos plated in MS media (Murashige & Skoog, 1962) regenerated into seedlings and with light exposure became green and also tillered. These seedlings were transplanted into peat pots and grew vigorously under greenhouse conditions. Root-tips collected from representative plants in peat-pots possessed the haploid n = 3x = 21 chromosome complement at mitosis with univalent meiosis (Figs. 1a and b) or n = 2x = 14 at mitosis also with univalent meiosis (Figs. 2a and b). It appears safe to conclude that embryos rescued from caryopses devoid of endosperm may not require cytological validation for establishing haploidy status. For colchicine doubling, seedlings were allowed to achieve vigorous growth prior to colchicine/DMSO treatment.

The final analytical stages: Embryo recovery frequencies ranged from 0 to 7.7% for durum cultivars except for 'Decoy' which had 12.3% (Table 1). Synthetic hexaploids derived from durums with 0% haploid production yielded n = 3x = 21 haploids between a 2.5 to 22.8% range; indicative of the influence of the D-genome of *Ae. tauschii* accessions. The overall haploid frequency range was between 2.5 to 42.6% for SH wheats. Most SH's out-performed their maternal durum parents in haploid production (Table 1). Data categories observed in the study were for florets pollinated, seed set, and embryos excised that led to the embryo formation percentage. Table 1 is structured to elucidate each durum and its related SH combination i.e., the durum cultivar (top line), followed by its SH derivative (bottom line). Embryo differentiation (not reported here) ranged from 50 to 70%; much less than the close to 90% which is the norm. Colchicine induced doubling was in congruence with the long-term average of reaching up to 95%. Some representative haploids of durum wheats and SH derivatives were not colchicine treated serving as the source for conducting meiotic analyses.

This study is indicative of the cumulative contribution of the seven chromosomes of the D genome viz., 1D to 7D towards haploid production. Inagaki *et al.*, (1998a) had analyzed the contribution of each D genome chromosome in a durum wheat where each homoeologue was substituted by its D genome chromosome (i.e. 1A substituted by 1D and 1B by 1D etc.) yielding 14 substitutions stocks that were analyzed. Observations supported the inference that chromosomes 1D, 3D, 4D and 7D contributed to higher frequencies of embryo formation with distinct specificity prevalent with the homoeologue durum chromosome. For example chromosome 3D substitution for 3A gave 0% embryos while 3D for 3B gave 11.1% embryos indicative of a cumulative role of the D and A or B genome chromosomes.

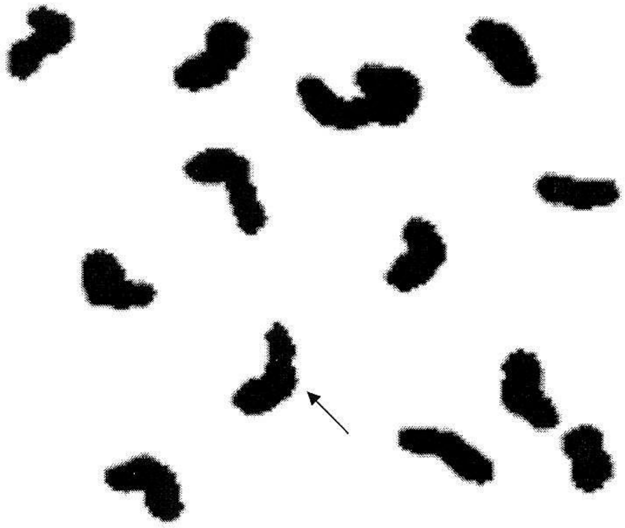
(a) ***T. aestivum*** **(n=3x=21, ABD)**



(b) ***T. aestivum*** **(21 univalents)**

Fig. 1. Cytological details of a bread wheat polyhaploid showing in (a) n = 3x = 21 chromosomes and in (b) univalent meiosis for these 21 chromosomes.

(a) *T. turgidum* (n=2x=14, AB)



(b) *T. turgidum* (19 univalents + 1 rod bivalent, arrowed)

Fig. 2. Cytological details of durum wheat polyhaploid showing in (a) n = 2x = 14 mitotic chromosomes and univalent meiosis in (b) with 12 univalents and 1 rod bivalent.

Table 1. Crossability of durum wheat (*Triticum turgidum*) cultivars and their synthetic derivatives with Z*ea mays* for production of polyhaploids recorded over embryo recovery percentage frequencies.

Durum wheat cultivars	Florets pollinated	Seed set	Embryos excised	Embryo formation (%)
Croc_1	168	54	0	0
Croc_1/*Ae. tauschii* (210)*	126	118	15	11.9
Arlin_1	168	19	1	0.6
Arlin_1/*Ae. tauschii* (665)	142	24	5	3.5
Rok/Kml	150	98	9	6.0
Rok/Kml/*Ae. tauschii* (214)	126	74	14	11.1
Altar 84	170	59	2	2.4
Altar 84/*Ae.tauschii* (224)	122	114	52	42.6
Dverd_2	154	10	1	0.7
Dverd_2/*Ae. tauschii* (214)	120	51	7	5.8
Laru	164	39	2	1.2
Laru/*Ae. tauschii* (309)	122	93	9	7.4
68.111/Rgb-u//Ward/3/Fgo/4/Rabi	144	124	5	3.5
68.111/Rgb-u//Ward/3/Fgo/4/Rabi/5/*Ae. tauschii* (191)	134	66	6	4.5
6973/Ward.7463//74110	186	134	0	3.2
6973/Ward.7463//74110/3/*Ae. tauschii* (665)	140	69	10	7.1
Cpi/Gediz/3/Goo//Jo/Cra	168	41	2	1.2
Cpi/Gediz/3/Goo//Jo/Cra/4/*Ae. tauschii* (637)	142	91	33	23.2
D67.2/P66.270	182	88	10	5.5
D67.2/P66.270//*Ae. tauschii* (211)	120	118	50	41.7
Cerceta	168	47	0	0.0
Cerceta/*Ae. tauschii* (742)	136	116	31	22.8
Sterna	150	85	4	2.7
Sterna/*Ae. tauschii* (446)	120	87	13	10.8
Rabi//Gs/Cra	176	31	2	0.0
Rabi//Gs/Cra/3/*Ae. tauschii* (190)	120	40	3	2.5
Sora	152	50	6	4.0
Sora/*Ae. tauschii* (192)	128	49	10	7.8
Scaup	164	31	1	0.6
Scaup/*Ae. tauschii* (493)	140	89	4	2.9
Snipe/Yav79//Dack/Teal	148	79	1	0.7
Snipe/Yav79//Dack/Teal/3/*Ae. tauschii* (528)	136	52	5	3.7
TK SN1081	158	121	3	1.9
TK SN1081/*Ae. tauschii* (222)	118	51	13	11.0
Yav_2/Tez	156	83	9	1.3
Yav_2/Tez//*Ae. tauschii* (435)	122	62	8	6.6
Yarmuk	162	13	0	0.0
Yarmuk/*Ae. tauschii* (217)	120	58	19	15.8
Decoy 1	146	100	18	12.3
Decoy 1/*Ae. tauschii* (188)	114	110	38	33.3
Garza/Boy	156	115	9	5.8
Garza/Boy/*Ae. tauschii* (271)	140	69	18	12.9
Araos	164	12	2	1.2
Araos/*Ae. tauschii* (269)	148	59	5	3.4

Table 1. (Cont'd.)

Durum wheat cultivars	**Florets pollinated**	**Seed set**	**Embryos excised**	**Embryo formation (%)**
Gan	158	100	2	1.3
Gan/*Ae. tauschii* (890)	138	40	20	14.5
Scoop_1	194	191	15	7.7
Scoop_1/*Ae. tauschii* (358)	120	86	25	20.8
Sty-us/Celta//Pals.3/Srn_5	142	53	0	0.0
Sty-us/Celta//Pals.3/Srn_5/4/*Ae. tauschii* (174)	132	74	9	6.8
Yav_3/Scot//Jo69/Cra/3/Yav79	168	31	0	2.4
Yav_3/Scot//Jo69/Cra/3/Yav79/4/*Ae. tauschii* (498)	128	57	7	5.5
Yar	124	38	6	7.3
Yar/*Ae. tauschii* (493)	132	110	23	17.4
68112/Ward	154	107	2	1.3
68112/Ward/*Ae. tauschii* (369)	134	21	2	1.5
Fgo/Usa2111	162	105	1	0.6
Fgo/Usa2111//*Ae. tauschii* (658)	116	76	19	16.4
Alg86/4/Fgo/Pales//Mexi_1/3/Ruff/Fgo/5/Ente	156	98	0	0.0
Alg86/4/Fgo/Pales//Mexi_1/3/Ruff/Fgo/5/Ente/6/ *Ae. tauschii* (518)	88	46	17	19.3
Botno	174	102	3	1.7
Botno/*Ae. tauschii* (617)	156	67	6	3.9
Cit71/Cpi	144	34	2	0.0
Cit71/Cpi//*Ae. tauschii* (629)	66	21	5	7.6
Lck59.61	172	40	2	0.6
Lck59.61/*Ae. tauschii* (173)	130	79	12	9.2
Trinakria	158	11	1	1.3
Trinakria/*Ae. tauschii* (700)	126	118	48	38.1
Rascon 37	164	113	4	2.4
Rascon 37/*Ae. tauschii* (312)	148	89	5	3.4
Ajaia_9	142	71	8	5.6
Ajaia_9/*Ae. tauschii* (330)	136	63	15	11.0
Scot/Mexi_1	186	28	0	0.0
Scot/Mexi_1//*Ae. tauschii* (314)	134	39	7	5.2
Falcin_1	140	103	4	4.3
Falcin_1/*Ae. tauschii* (414)	128	114	15	11.7
Shag 22	112	53	0	0.0
Shag 22/*Ae. tauschii* (227)	144	98	7	4.9
Kapude_1	112	100	1	0.9
Kapude_1/*Ae. tauschii* (341)	138	100	5	3.6
Chen 7	118	29	0	0.0
Chen 7/*Ae. tauschii* (429)	108	89	20	18.5
Aconchi 89	180	0	0	0.0
Aconchi 89/*Ae. tauschii* (290)	128	43	6	4.7
Alcatraz	152	16	6	2.6
Alcatraz/*Ae. tauschii* (398)	136	100	27	19.9
Local Red	128	39	4	1.6
Local Red/*Ae. tauschii* (449)	92	30	4	4.4

**Ae. tauschii* accession number in CIMMYT Wheat Wide Crosses working collection.

Applications: The data showing durum cultivar specificity for haploid production suggests that we are still a long way from having this protocol integrated into a durum wheat breeding program to enhance its efficiency as is the case for bread wheat breeding (Inagaki *et al*., 1998b; Mujeeb-Kazi *et al*., 2002). The success reported via high fertilization frequencies on a limited number of durum genotypes (O'Donoughue & Bennett, 1994a) is encouraging and needs to be studied in greater depth. It is also encouraging that in our study some high quality cultivars also respond to maize based haploid induction (Table 1). Hence these cultivars could form a basis for exploitation in molecular mapping, cytogenetics and alien gene transfer programs (Mujeeb-Kazi, 2005) where doubled haploidy is considered advantageous by several researchers. Durum wheats have inadequate genetic diversity for some important production constraints for which presently *Fusarium* head scab apparently is the priority. D genome based SH wheats have good resistance, and it is plausible to transfer the *Ae. tauschii* resistance into high quality durum wheats that are amenable to haploid generation. The strategy involves the *ph1c* Capelli genetic stock to initiate D and A genome exchanges by homoeologous recombination, identifying the D introgression derivatives, top-crossing with elite durums positive for haploid induction and achieving homozygosity of the translocated derivatives. The above protocol is adapted from Mujeeb-Kazi (2001) currently in use for bread wheat germplasms. The durums identified in Table 1 as being amenable to haploid induction are good candidates for use in development of molecular mapping populations where mostly F1 seed from resistant x susceptible crosses are utilized, having at least the trait susceptible durum cultivar responsive to haploidy. Such populations are routinely produced for bread wheats and we are optimistic that a similar approach may function for durums instead of the conventional method of developing recombinant in-bread lines *via* single seed descent.

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