Phenotypic and molecular prospection of reduced height sunflower germplasm

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ABSTRACT

- Decreasing plant height and increasing stem diameter may be useful to ameliorate standability of sunflower. Lodging or stem breakage due to adverse growing conditions can reduce yield significantly in some years. Strong winds in conjunction with excessive precipitation and saturated soil conditions can cause up to 100% crop loss. However, progress in improving the standability of conventional height sunflower has been slow. Therefore, the potential of reduced height germplasm as a strategy to increase yield potential and reducing stem lodging has been recognized during the past three decades but still deserves to be fully explored in sunflower. Three sources of reduced height with an equal or similar number of leaves as conventional-height sunflowers were reported: 'DDR', 'Donsky' and 'Donskoi 47'. DDR and Donsky were used to develop several restorer and maintainer public lines. The inheritance of reduced plant height in lines tracing back to 'Donsky' was reported to be controlled by alleles at two loci. Reduced height in 'Donskoi 47', on the other hand, is controlled by a single dominant gene. Inheritance of reduced height in the source 'DDR' is controlled by only one factor which was recently mapped in the sunflower genome. A candidate gene for this factor, *Della1*, was postulated based on co-segregation and sequence analysis. A SNP marker, Della1-143, was designed as an allele specific marker of the reduced height factor present in 'DDR'. The objectives of this work were to phenotypically characterize under the same environmental conditions a set of 30 genetic materials reported to have reduced height and to determine if they carry the Della1-143 allele.
- Thirty inbred lines or open-pollinated populations (OPPs) reported to have reduced height were obtained from GRIN and other sources. Fifty plants each of these genetic materials were sown under field conditions at Nidera Experimental Station, Venado Tuerto, Argentina, together with conventional height inbred lines used as checks. At flowering, plant height, stem diameter, internode length and days to flowering were recorded on each plant. Leaf tissue of each plant was also collected for DNA analysis. PCR–based markers were used to detect single nucleotide polymorphisms diagnostic of the *Della1* allele of 'DDR'.
- Fifteen out of the 30 genetic materials (12 inbred lines and 3 OPP) showed at least one plant with reduced height, thick stem, and short internode length. In the case of the inbred lines, this reduced-height syndrome was homogenously expressed by all the plants and in the case of the OPPs only one to four plants expressed it. These plants were selfed and the syndrome was fixed in the descendants. Days to flowering in these 12 lines as well as in the reduced height plants of the OPPs materials fell in the interval range of the conventional checks used as control. Five out of the 15 reduced-height materials traced back to 'Donsky'. Molecular characterization indicated that 10 out of the 15 reduced-height genotypes presented the 'DDR' codon modification at the *HaDella1* gene sequence. These 10 genotypes were inbred lines and half of them traced back to the source 'Donsky'. None of the conventional height plants analyzed showed the SNP Della1-143 allele.
- Phenotypic and molecular characterization allowed us to determine that the same allele at *Della1* locus, involved in the regulation of gibberellic acid (GA) metabolism, is responsible for the reduced height syndrome occurred in 'DDR', 'Donsky' and some other unknown sources. On the other hand, other SNPs in the sunflower *Della* genes, mutations in the genes of the GA and brassinosteroid metabolic pathways might be responsible for the reduced height syndrome observed in five other lines and OPPs.
- In order to fully exploit the reduced height trait as a character to maximize sunflower yield potential in certain environmental conditions, it is necessary to make available, understand and evaluate all the physiological mechanisms involved in controlling the trait and their genetic nature. This work combined phenotypic and molecular characterizations in order to partially accomplish these goals. Research into the genetic basis of the reduced height syndrome other than *Della1* gene polymorphisms are in progress.

Key words: DELLA, dwarf, genetic resources, gibberellic acid, reduced height,

INTRODUCTION

Root and stem lodging, defined as the permanent displacement of the stem from its vertical position, cause important yield losses in sunflower (*Helianthus annuus*). The prostrate head of lodged plants are not retrieved during mechanical harvesting causing significant losses. Also, lodging may contribute to fixing the upper limit to commercially viable crop population density, since yield is known to increase up to densities higher than those currently used (Hall et al., 2010). Progress in improving the standability of conventional height sunflower has been slow (Miller and Fick, 1997). Current hybrids, when protected from lodging and disease, show increases in yield potential with crop population density up to densities that are almost three times the current commercial density of 5 plants m^{-2} (López Pereira et al., 2004), it seems very likely that propensity to lodge at high crop population densities also plays a part in reducing realizable yield potential in this crop (Hall et al., 2010). Therefore, reduced height germplasm has the potential to increase stem strength and also yield potential of the sunflower crop. Decreasing plant height and increasing stem diameter may be useful in increasing standability of sunflower. Therefore, the potential of reduced height germplasm as a strategy to increase yield potential and reducing stem lodging has been recognized during the past three decades but still deserves to be fully explored in sunflower (Sala et al., 2012).

Reduced height in sunflower controlled by recessive genes in lines with a reduced number of leaves has been reported by Vranceanu (1974), Fick (1978), Berretta de Berger (1984), Berretta de Berger and Miller (1985), Cecconi et al. (2002), Jagadeesan et al. (2008) and Fiambrini et al. (2011). In particular, *dwarf1* (*dw1*, Cecconi et al. 2002) and *dwarf2* (*dw2*, Fiambini et al. 2011) are severe dwarf mutants affecting vegetative and reproductive growth which can be restored to the wild type phenotype by exogenous gibberellic acid (GA) applications. Nevertheless, none of them have been used to improve yield as yet because of the excessively severe phenotypes of these mutants. Three sources of reduced height with an equal or similar number of leaves as conventional-height sunflowers were reported: 'DDR', 'Donsky' and 'Donskoi 47'. DDR and Donsky were used to develop several restorer and maintainer lines (Miller and Gulya, 1989; Miller 1993; Velasco et al., 2003a). The inheritance of reduced plant height in the sunflower line Dw89, which trace back to 'Donsky', was reported to be controlled by two recessive alleles, designated *dw1* and *dw2* (Velasco et al., 2003b). Reduced height in Donskoi 47, on the other hand, is controlled by a single dominant gene, *Dw* (Tolmachov, 1991). Inheritance of reduced height in the source 'DDR' is controlled by only one factor, *Rht1*, which was recently mapped in the sunflower genome (Ramos et al., 2012). A candidate sequence for this factor, *HaDella1*, was postulated based on co-segregation and sequence analysis. A SNP marker, Della1-143C, was designed as an allele specific marker of the reduced height factor present in 'DDR' (Ramos et al., 2012). The objectives of this work were to phenotypically characterize under the same environmental conditions a set of 30 genetic materials reported to have reduced height and to determine if they carry the Della1-143C allele.

MATERIALS AND METHODS

Thirty inbred lines or open-pollinated populations (OPPs) reported to have reduced height were obtained from GRIN and other sources. These genetic materials were sown under field conditions at Nidera Experimental Station, at Venado Tuerto, Argentina, together with four conventional height inbred lines used as checks (Table 1). Each line was sown by hand in three rows 70 cm apart and 25 cm among plants. At R5.1 stage of development (Schneiter and Miller, 1981) measurements were recorded on 10 plants in full competence for the following characters: days to flowering, plant height, stem diameter. Days to flowering are the number of days from emergence (VE stage) to R5.1; plant height was taken as the distance between the cotyledonal node and the point where the stem is attached to the capitulum. Stem diameter was measured between the 3rd and 4th true leaves.

Leaf tissue of five plants for each inbred line or OPP and from the reduced height plants observed in the OPP were collected for DNA analysis. Haplotypification of these materials for the *Hadella1* sequence was assessed by the SNP markers Della1-143C/T (Ramos et al., 2012).

RESULTS

Fifteen out of the 30 genetic materials (12 inbred lines and 3 OPPs) showed at least one plant with reduced height, thick stem, and short internode length. In the case of the inbred lines, this reduced-height syndrome was homogenously expressed by all the plants and in the case of the OPPs only one to four plants expressed it (Table 1). These plants were selfed and the syndrome was fixed in the progeny.

Table 1. Phenotypic characterization and haplotype for HaDella1 sequence for thirty genetic materials and four conventional checks.

Genetic material	Accesion	Type of genetic material[1]	N° of plants analyzed	Days to flowering	Height [2] (cm)	N° of plants with reduced height	Stem diameter (mm)	Internode length (cm)	Haplotype for HaDella1[3]
RHA360	PI 531073	IL	50	64 ± 2	53.4 ± 6.1	50	27.7 ± 2.7	2.3 ± 0.2	C
RHA361	PI 531074	IL	47	64 ± 2	48.5 ± 3.5	47	28.5 ± 2.7	2.4± 0.2	C
RHA362	PI 531075	IL	44	62 ± 3	55.8 ± 4.8	44	25.5 ± 5.8	2.5 ± 0.3	C
DW89	PI 631495	IL	49	72 ± 1	44.2 ± 4.8	49	20.7 ± 0.7	1.6 ± 0.2	C
DW271	PI 631496	IL	44	71 ± 2	61.6 ± 2.9	44	24.3 ± 4.3	2.9 ± 0.2	C
PI386316	PI 386316	OPP	45	62 ± 2	65 ± 4.8	45	19.6 ± 2.1	4.1 ± 0.5	T
CM-619	PI546356	IL	50	56 ± 3	96 ± 4.5	0	20.2 ± 4.0	4.7 ± 0.2	T
Karlick	PI 650558	OPP	38	62 ± 3	76.25 ± 21.6	0	20.1 ± 6.3	4.9 ± 1.0	T
Chermianka 73	PI 650555	OPP	44	62 ± 3	107.3 ± 17.7 (68-70)	2	27.8 ± 4.9	4.9 ± 0.8	T
VIR 2321	PI 386323	OPP	42	60 ± 2	70.4 ± 12.3	0	27.7 ± 5.6	3.4 ± 0.1	T
VIR 1903	PI 386236	OPP	50	53 ± 3	73 ± 10.8	0	21.8 ± 3.8	4.3 ± 1.5	T
Volgar	PI 371939	OPP	43	57 ± 2	140.2 ± 18.9	0	19.7 ± 7.5	7.7 ± 0.7	T
Donsky	PI 307935	OPP	39	63 ± 3	146.5 ± 12.8	0	19.5 ± 3.4	6.0 ± 0.4	T
Bamanslkij	PI 291410	OPP	44	57 ± 3	110.4 ± 17.4	0	26.7 ± 1.8	6.2 ± 1.2	T
Szaratovskij Ranni	PI 291404	OPP	42	61 ± 2	126.6 ± 23.5	0	27.8 ± 7.9	5.9 ± 1.6	T
Oleifeira	PI 284862	OPP	42	53 ± 2	86.7 ± 11.8	0	26.3 ± 1.8	4.8 ± 0.7	T
Tchernyanka 66	PI 265104	OPP	35	59 ± 2	110.2 ± 18.9 (58-60)	2	24.1 ± 7.3	5.0 ± 0.6	T
POL 234605	Ames 25949	OPP	40	63 ± 3	120 ± 18.4	0	24.4 ± 4.6	6.4 ± 1.1	T
CM 632	PI 566830	OPP	42	63 ± 3	86 ± 10.1	0	19.6 ± 4.6	3.9 ± 0.7	T
PHA009	PI 601368	OPP	36	64 ± 2	83.0 ± 3.4	0	15.7 ± 2.2	4.6 ± 0.6	T
VIR 847	PI 386230	OPP	38	52 ± 2	88.8 ± 13.3	0	25.5 ± 2.9	6.2 ± 0.4	T
B4268	PI 650391	OPP	37	71 ± 3	146.8 ± 20.1	0	22.3 ± 2.8	6.1 ± 0.8	T
Sunspot	-	IL	46	65 ± 1	37.3 ± 9.7	46	16.4± 2.6	1.7 ± 0.2	C
Irish Eyes	-	IL	48	67 ± 1	49.6 ± 3.2	48	23.6 ± 2.1	1.6 ± 0.3	C
Teddy Bear	-	IL	49	61 ± 3	40.5 ± 4.4	49	24.8 ± 3.0	1.6 ± 0.1	T
Choco Sun	-	IL	36	70 ± 2	39.3 ± 2.3	36	22.0 ± 1.4	1.4 ± 0.1	C
Little Dorrit	-	F_1 Hybrid	46	70 ± 1	53.8 ± 6.8	46	23.5 ± 4.6	1.9 ± 0.1	C
Music Box	-	IL	40	60 ± 2	39.8 ± 4.6	40	14.1 ± 1.2	1.5 ± 0.2	T
Double Shine	-	F_1 Hybrid	38	63 ± 2	112.8 ± 14.8	0	25.8 ± 4.0	3.5 ± 0.6	T
Yellow Spray	-	IL	46	66 ± 1	48.2 ± 4.1	46	21.8 ± 2.6	1.7 ± 0.1	C
Conventional checks									
HA89	PI 599773	IL	50	62 ± 2	80.8 ± 7.0	0	22.3 ± 4.2	3.4 ± 0.5	T
HA821	PI 599984	IL	48	63 ± 1	107.2 ± 3.6	0	23.1 ± 2.0	3.8 ± 0.3	T
RHA271	PI 599786	IL	46	64 ± 2	119.8 ± 9.9	0	22.2 ± 4.3	5.5 ± 0.8	T
RHA274	PI 599759	IL	50	54 ± 2	112.0 ± 12.1	0	23.2 + 4.6	6.3 ± 0.7	T

1 IL = Inbred line

2. In parenthesis, the height of reduced height plants of the OPP

3. 'C' corresponds to the haplotype from the reduced height source 'DDR'.

Days to flowering in these 12 lines as well as in the reduced height plants of the OPPs fell in the interval range of the conventional checks used as controls, indicating that the reduced height in this plants is not associated with earliness.

Molecular characterization indicated that 10 out of the 15 reduced-height genotypes presented the 'DDR' codon modification at the *HaDella1* gene sequence. These 10 genotypes were inbred lines and half of them traced back to the source 'Donsky' (RHA360, RHA361, RHA362, DW89 and DW271). None of the conventional height plants observed showed the SNP Della1-143C which characterizes the reduced height factor derived from 'DDR' (Table 1).

DISCUSSION

There are various factors responsible for dwarfism in plants, but GA and brassinosteroid (BR) are the most intensely studied in determining plant height (Fujioka and Yokota 2003; Yamaguchi 2008). GAs are essential phytohormones that regulate many aspects of plant growth and development, including seed germination, leaf expansion, stem and root extension, flower induction and development, seed development, and fruit expansion (for review, see Fleet and Sun 2005; Olszewski et al. 2002; Swain and Singh 2005; Yamaguchi 2008).

Dwarf mutants deficient in endogenous GAs have been described for several plant species (for review, see Sakamoto et al., 2004; Yamaguchi, 2008). In sunflower three dwarf mutants have been reported (*dw1*: Cecconi et al., 2002; Jambhulkar, 2002, *dw2*:Fiambrini et al., 2011). These mutants displayed abnormal development of flower organs without seed development. GA treatments were able to revert *dw1* and *dw2* to wild type or near wild type phenotype, although severe aberrations of reproductive organs were retained (i.e. precocious abort of pollen; Cecconi et al., 2002). The most obvious alterations of *dw2* plants were the lack of stem growth, reduced size of leaves, petioles and flower organs, and retarded flower development. Pollen and ovules were produced but the filaments failed to extrude the anthers from the corolla (Fiambrini et al., 2011). In contrast to these mutants, *Rht1* from DDR is defective in the GAs response pathway (Ramos et al., 2012). This pathway is controlled by the DELLA repressors, which are characterized by their N-terminal DELLA domain (Pysh et al., 1999). Characteristically, this type of mutants encodes an altered form of the DELLA protein that are resistant to GAs-induced degradation and constitutively blocks GAs signaling (Peng et al., 1999). DELLA proteins encoded by GAI (Peng et al., 1997) and RGA (Silverstone et al., 1998) in *Arabidopsis*; *d8* in maize (*Zea mays*, Winkler and Freeling, 1994); VvGAI in grape (*Vitis vinifera*, Boss and Thomas, 2002); SLN1 in barley (*Hordeum vulgare*, Chandler et al., 2002), SLR1 in rice (*Oryza sativa*, Ikeda et al., 2001, Itoh et al., 2002), BnRGA in rapessed (*Brassica napus*, Liu et al., 2010), *B. rapa* BrRGA1 (Muangprom et al., 2005) and LeGAI in tomato (*Solanum licopersicum*, Bassel et al., 2004) have been isolated and have conserved functions as GA signaling repressors.

Phenotypic and molecular characterization allowed us to determine that the same haplotype at *HaDella1* sequence, involved in the regulation of GA metabolism, is responsible for the reduced height syndrome occurred not only in 'DDR', but also in 'Donsky' and some other unknown sources. On the other hand, other SNPs in the sunflower *Della* genes, mutations in the genes of the GA and BR metabolic pathways might be responsible for the reduced height syndrome observed in five other lines and OPPs.

In order to fully exploit the reduced height trait as a character to maximize sunflower yield potential in certain environmental conditions, it is necessary to make available, understand and evaluate all the physiological mechanisms involved in controlling the trait and their genetic nature. This work combined phenotypic and molecular characterizations in order to partially accomplish these goals. Research into the genetic basis of the reduced height syndrome other than *Della1* gene polymorphisms are in progress.

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