J Appl Genetics (2011) 52:35–38
DOI 10.1007/s13353-010-0002-4

PLANT GENETICS • SHORT COMMUNICATION

Inheritance and molecular mapping of a gibberellin-insensitive dwarf mutant in groundnut (*Arachis hypogaea* L.)

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Received: 3 August 2010 / Revised: 30 September 2010 / Accepted: 6 October 2010 / Published online: 3 November 2010
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Abstract A dark green dwarf mutant, TGM 167, was isolated from a gamma ray + sodium azide mutagenised population of cultivated groundnut breeding line, TFDRG 5. The mutant had a 45.8% reduction in height due to its shorter internodal length. Further, it was found to be insensitive towards exogenous GA_3 application, although it had nearly the same level of endogenous GA_3 as the parent. Genetic analysis revealed that the dwarfism is under the control of a single dominant gene. This dominant dwarfing gene was mapped with an SSR marker TC3H02 at a distance of 9.7 cM.

Keywords *Arachis hypogaea* · Dwarf mutant · Gamma rays · Gibberellin · Sodium azide · SSR markers

Reduced plant height is an important agronomic trait associated with yielding capacity and lodging resistance in crop plants. A large number of dwarf mutants were isolated in different crop species and several of them have been characterised as gibberellic acid (GA) deficient or insensitive mutants. Either the disruption or abnormal synthesis of GA or the disruption of GA signal transduction can lead to dwarfism (Ueguchi-Tanaka et al. 2000). A common feature of GA-sensitive mutants is that they are recessive and deficient for GA because of a block in their biosynthetic pathway (Phinney 1984). Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown extensively in subtropical regions. Groundnut dwarf mutants were induced using X-rays (Patil 1966), gamma rays (Patil and Mouli 1978; Badigannavar and Mondal 2010), laser (Bozhan et al. 1997), diethyl sulfate (Ashri 1970), ethyl methane sulfonate (Gowda et al. 1996), ethidium bromide (Levy and Ashri 1975) and colchicine (Tiwari and Khanorkar 1984). Although enough information is available for the isolation of induced mutants in groundnut, their characterisation at biochemical and molecular levels is lacking. The present study reports on the isolation of a dominant dwarf mutant in groundnut and its preliminary biochemical and molecular characterisation.

Dry seeds (8–10% moisture) of TFDRG 5 (*A. hypogaea* ssp. *fastigiata* var. *vulgaris*) were treated with gamma rays (200 and 300 Gy) and/or sodium azide (NaN_3 1, 2 and 3 mM) singly or as a combination of both (Badigannavar et al. 2005; Mondal et al. 2007). The M_2 population having 20,619 plants was grown in the field during the rainy season (June to September) of 2004 and the dwarf mutant, TGM 167, was isolated along with other morphological and yield-related mutants and tested for their true breeding behaviour in the M_3 and subsequent generations. TGM 167 belongs to botanical type *A. hypogaea* ssp. *fastigiata* var. *vulgaris*, like its parent. Further, all of the true breeding mutants, including TGM 167, were evaluated for their agronomic performances in the M_8 (rainy season 2007) and M_9 (summer 2008) generations and the agronomic data were analysed using IRRISTAT 2.0 software (International Rice Research Institute [IRRI] 2003).

The dwarf mutant TGM 167 was crossed with TFDRG 5. Segregation data for plant height in the F_2 and F_3 plants were subjected to the χ^2 test for goodness of fit. A total of 1,114 published SSR primer pairs were screened between the parent and dwarf mutant. The SSR amplification, detection and data scoring were as described by Mondal and Badigannavar (2010). The polymorphic primer pairs were used for the genotyping of 73 F_2 plants derived from TFDRG 5 × TGM 167 and TGM 167 × TFDRG 5 crosses.

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Kosambi map distances (Kosambi 1944) were estimated through linkage analysis between plant height segregation in the F_2 population and SSR marker genotyping data using MAPMAKER version 3.0b (Lincoln et al. 1993) with a minimum LOD of 3.0.

Bioactive GA_3 (Sigma, St. Louis, MO, USA) was dissolved in a small amount of absolute ethanol and diluted with distilled water. A field experiment was carried out by spraying 0, 10, 20, 40, 60, 80 and 100 ppm GA_3 over the mutant in order to detect GA responsiveness. After 10 days of planting, the mutant and parent were sprayed daily until they were moist for a week in three replications, each consisting of ten plants. The height was measured from 40-day-old plants and compared with the untreated parent and mutant. In in vitro studies, surface-sterilised seeds (0.1% $HgCl_2$) of the mutant and parent were placed in conical flasks having Murashige and Skoog media + 1% agar–agar (Murashige and Skoog 1962) with and without GA_3 at 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M in three replications. Each replication consisted of five flasks having four plants. The plants were grown at a 12/12-h day/night cycle and an average temperature of 28°C. The plant height was measured after 30 days. GA_3 responsiveness for lipase production was studied in groundnut by mimicking the studies of α amylase activity in embryo-less seed cotyledons in cereals. Hence, the lipase activity was chosen over α amylase as the groundnut is an oilseed crop and the main storage products are triglycerides (oils) rather than starch. Four to five embryo-less quarter cotyledons per plate were surface-sterilised, washed and positioned perpendicularly on a tributyrin agar plate (5% tributyrin and 1.5% agar at pH 7.0) with or without GA_3 (10^{-3} to 10^{-5} M). The GA plates were prepared by adding filter-sterilised GA_3 solution to cold medium. The plates were checked for the clear zone of lipid hydrolysis after seven days of incubation at 28± 2°C in the dark. The quantification of endogenous GA_3 was performed by gas chromatography fitted with a flame ionisation detector (GC 2014, Shimadzu, Kyoto, Japan). The apical meristem portion with two pairs of leaves was taken from 60-day-old plants of parent and mutant for GA_3 extraction. The apical portion of the stem was ground to a fine powder in a mortar and pestle with liquid N_2 and GA_3 was extracted as per the method described by Reddy et al. (1989) for groundnut. Later, the extracts were esterified by the saponification–transesterification method using 0.5 N NaOH in methanol and 10% BF_3 in methanol and were injected into gas chromatography apparatus. The gas chromatography chamber is equipped with an automated sampler, injector and a BP 1 (25-m) capillary column (SGE Analytical Science Pvt. Ltd., Victoria, Australia) coated internally with a thin film (0.25 µm diameter) of 100% dimethyl polysiloxane. The initial column temperature was 100°C and was held for 1 min, followed by an increase to 265°C at the rate of 25°C/min. The injector and detector temperature were set to 320 and 300°C, respectively. Gas flow rates of 3.5 (column flow), 30 and 300 ml/min for N_2, H_2 and air, respectively, were maintained. The GA_3 content in the mutant and parent was identified and quantified by comparing the retention time of standard GA_3 (Sigma, St. Louis, MO, USA) under the same temperature conditions and gas flow rates.

In the present study, a dwarf mutant TGM 167 was isolated from the treatment of 200 Gy gamma rays + 1 mM NaN_3 with a mutation frequency of 4.85×10^{-5}. In the M_3 generation, dwarf plant bred true with a significant reduction in height as compared to the parent. The dwarf phenotype was maintained in TGM 167 from seed emergence until maturity. The plant height in TGM 167 was reduced by 45.8% as compared to TFDRG 5 (Table 1). Although there was no reduction in the number of internodes on the main axis in the mutant and parent, the internodal length was considerably shortened in the mutant (Table 1). Similar pod yield and shelling percentage with slightly reduced seed size was observed in TGM 167 as compared to TFDRG 5. All of the F_1 plants from both direct and reciprocal crosses between the parent and mutant were dwarf, indicating the absence of involvement of cytoplasmic factors. The F_2 plants were segregated as 3 dwarf:1 normal height. The segregation in the F_3 plants was 1 (all dwarf):2 segregating (3 dwarf: 1 normal height):1 (all normal height) (Table 2). Both phenotypic and genotypic segregation revealed that dwarf trait in the mutant was conditioned by a single dominant gene. Most of the earlier studies reported that genes for dwarf mutants in groundnut were recessive (Ashri 1968; Patil and Mouli 1975; Bhuiyan 1984), dominant with recessive lethal effect (Ashri 1970) and incomplete dominant (Branch and Hammons 1983;

Table 1 Agronomic performances of the dwarf mutant TGM 167 in M_8 and M_9 generations as compared with TFDRG 5

Traits	(Parent)		(Mutant)	
	2007	2008	2007 (M_8)	2008 (M_9)
Plant height (cm)	46.1	39.3	25.7*	20.6*
Number of internodes	21.3	19.0	20.8	17.8
Internodal length (cm)	2.16	2.07	1.23*	1.15*
Number of branches	8.4	7.9	10.2	10.2*
Pod weight/plant (g)	20.8	21.3	23.5	21.2
Seed weight/plant (g)	15.2	15.4	16.8	15.1
Shelling outturn (%)	73.5	72.3	71.4	71.1
100 kernel weight (g)	48.7	50.3	40.3*	45.4*

*Significantly different from the parent at p=0.05

Table 2 Inheritance of dwarfness in F_2 and F_3 plants of reciprocal crosses between the dwarf mutant TGM 167 and the parent TFDRG 5

Generation	Number of progenies	Number of dwarf plants	Number of normal plants	χ^2 (df)	*p*-value
TFDRG 5 × TGM 167					
F_1	–	2	–		
F_2	2	26	8	0.04 (1)	0.84
F_3	10	194	0		
	16	345	115	0.00 (1)	1.00
	8	0	262		
	Genotypic segregation (1:2:1)			0.35 (2)	0.84
TGM 167 × TFDRG 5					
F_1	–	2	–		
F_2	2	31	8	0.42 (1)	0.52
F_3	12	297	0		
	19	329	112	0.04 (1)	0.84
	8	0	275		
	Genotypic segregation (1:2:1)			0.85 (2)	0.65
Pooled F_2	4	57	16	0.37 (1)	0.54
Homogeneity				0.09 (1)	0.76

Badigannavar and Mondal 2010). This is the first known report of a dominant dwarf mutant in groundnut.

The molecular mapping of agronomically important traits in groundnut is very limited. Lower molecular polymorphism is a common feature in cultivated groundnut due to a recent event of monophyletic origin followed by genome duplication (Moretzsohn et al. 2005). In our study, the screening of 1,114 published SSR markers between the parent and mutant revealed that only seven (0.63%) (TC3H02, TC3E02, TC11H06, IPAHM 659, IPAHM 468, EM 31 and pPGPseq_2H08) were polymorphic. The very low SSR polymorphism (0.63%) between the parent and mutant was expected, as induced mutants have nearly identical genetic architecture to the parents. The genotyping of F_2 plants with all of these polymorphic SSR primers detected an expected 1:2:1 segregation for two markers (TC3H02 and IPAHM659) and the others had distorted ratios. Linkage analysis mapped TC3H02 at 9.7 cM from the dwarf locus in the mutant. The marker TC3H02 had also been mapped earlier to group I of the AA genome map (Moretzsohn et al. 2005) and linkage group Lg 5 in the SSR-based tetraploid linkage map (Hong et al. 2010) of groundnut.

Most of the plant species have the bioactive gibberellins in the form of GA_3 and GA_4. Previous reports in groundnut explained that 10 ppm GA_3 was sufficient to induce height increment in a dwarf mutant with variable penetrance and expressivity (Ashri 1970). The spraying of GA_3 (10, 20, 40, 60, 80 and 100 ppm) on the apical meristem of seedlings of TGM 167 (5.0–5.2 cm) showed no response in plant height increment as compared to untreated mutant (5.0 cm) and treated or untreated parent (12.5 cm; LSD=0.32 at p=0.05). Moreover, the mutant plants did not respond to the medium containing GA_3 at different concentrations (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M) under in vitro study. Further, there was no differential response even at 10^{-3} M (346 ppm) GA_3 on lipase production in both the parent and mutant compared to the plate without GA_3. Of the multiple peaks obtained using standard GA_3 in gas chromatography, the major peak was observed at 9.5 min. The quantitative analysis revealed that the GA_3 content in the mutant (132±7 ng/g) was on a par with its parent (130±11 ng/g of fresh weight). A similar phenomenon of accumulation of the same amount of native gibberellins in both the parent and mutant (*D8*) in maize explained a mutation in GA receptor that controls a product downstream from the binding of the bioactive GA to a receptor (Fujioka et al. 1988). Several such dominant dwarf mutants were reported in wheat and maize wherein mutation was detected in the orthologue of the *Arabidopsis* gibberellic acid-insensitive (*GAI*) gene.

Acknowledgement Our special thanks go to Shri R. K. Sachan for the help in the field experiments.

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