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Morphological mutants of St. Augustinegrass induced by gamma ray irradiation

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Abstract

St. Augustinegrass is a widely used turf and pasture grass in the southern US. 'Raleigh' is a cultivar known for superior cold tolerance than other St. Augustinegrass cultivars. However, its coarse-leaf texture and long internodes are undesirable when planted in home lawns. Mutagenesis by gamma ray irradiation was employed to treat node cuttings and calli for inducing semi-dwarf growth phenotype. Dosages of 48.5 and 72.6 Gy were determined as LD_{50} and LD_{20} for the cuttings, respectively. Regeneration ability of callus was greatly reduced when irradiated with higher dosages (over 100 Gy). Thirteen morphological mutants were identified among over 3000 node cuttings and 80 pieces of calli treated. Most mutants were semi-dwarf type with reduced internode length and leaf blade length. One mutant had much less and shorter stolons and displayed an upright and tufty growth pattern. The altered morphological traits were stable as shown by their growth performance in various locations and conditions.

Key words: Gamma ray irradiation — mutagenesis — semi-dwarf — St. Augustinegrass

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is widely used as a home lawn grass, thriving throughout the southeastern US from Texas to Florida and as far north as the coastal areas of North Carolina and Virginia because its superior shade tolerance and stoloniferous growth habit (Busey 2003). 'Raleigh' is a cultivar known for its superior cold tolerance over many other St. Augustinegrass cultivars. It was collected from a home lawn in Raleigh, North Carolina, and developed by Dr W.B. Gilbert at North Carolina State University in the early 1980s. However, like many other St. Augustinegrass cultivars, the coarse-leaf texture and long internodes are often undesirable when planted in home lawns. The sod industry of North Carolina has a demand for a cultivar, which has improved semi-dwarf growth characteristics.

Irradiation mutagenesis has been applied for turfgrass improvement, in which 10 turfgrass cultivars have been developed and released (FAO/IAEA database 2006), e.g., 'TifBlair' centipedegrass [*Eremochloa ophuiroides*] (Hanna et al. 1997) and 'TifEagle' bermudagrass [*Cynodon dactylon* (L.) Pers.] (Hanna and Elsner 1999). Each mutant showed finer texture and superior turf quality. In St. Augustinegrass, Busey (1980) investigated dosage effects of gamma ray from ^{137}Ce on stolons of seven genotypes of the grass. Although 45 Gy was found as an optimum dosage to induce mutations for most genotypes, 'Bitterblue' and another accession were entirely killed at 40 Gy. Powell and Toler (1980) treated over 2600 nodes of 'Floratum' at a dosage of 58.3 Gy and later released two mutant cultivars, 'TXSA 8202' and 'TXSA 8212' (Toler et al. 1985) with multiple disease resistance against *Magnaporthe, Ceratosphaeria, Scherophthora* and *Tanatephorus*. However, there is no report on mutagenesis of 'Raleigh' by gamma ray irradiation for cultivar development.

The purpose of this research is to induce semi-dwarf mutants from 'Raleigh', which still show good growth vigour, for cultivar improvement, and further characterize them in the field conditions.

Materials and Methods

Irradiation dosage effect on single node cuttings and calli: Dosage effect of gamma ray from ^{60}Co was investigated on single node cuttings and calli induced from tissue culture. For single node cuttings, stolons were randomly collected from a field plot at North Carolina State University (NCSU) Lake Wheeler Turf Field Laboratory, Raleigh, NC, USA and the attached soil was washed off with tap water. The stolons were cut into single node cuttings. Shoots and roots were trimmed to 3–4 cm in length so that the cuttings would have similar size in irradiation treatment. The cuttings were sealed in plastic bags and irradiated with various dosages of gamma ray from ^{60}Co with a rate of approximately 80 Gy/h, at a facility in the Nuclear Engineering Department, NCSU. The control is the cutting which was not irradiated, but kept in plastic bags and sprayed with water to maintain moisture. The cuttings were then planted to Metro-Mix-200 soil (Scotts, Marysville, OH, USA) for recovery in the greenhouse at 25 ± 5°C.

In a preliminary dosage effect experiment, the cuttings were treated with dosages of 0, 15, 45, 100, 200, 400, 800 and 1600 Gy, respectively, with 20 cuttings per treatment. To measure the dosage effect, the fresh weight of the cuttings before the treatment and the fresh weight 6 weeks later were recorded for each treatment. Growth index was calculated as the final fresh weight minus the initial fresh weight divided by the initial weight.

Following the preliminary experiment, a replicated experiment was performed to expose the cuttings to a narrower range of dosages, that is, 0, 50, 60, 70, 80, 90 and 100 Gy, respectively, to determine the dosages for LD_{50} and LD_{20} (survival rates of 50% and 20%, respectively). Each replicate had 20 cuttings. Three replications were included in the experiment. Survival of the cuttings was scored 6 weeks later. Survival rate was calculated as the percentage of the number of cuttings with re-growth divided by the number of total cuttings treated. Probit analysis (Finney 1971) was conducted to

determine LD_{50} and LD_{20} using SAS program (Ver. 9.1, SAS Institute 2003).

To induce mutations from calli, five dosages (0, 25, 50, 100 and 200 Gy) were applied. Calli were induced from immature embryos of 'Raleigh' on the MS basal medium supplemented with 30 g/l sucrose, 3.2 g/l phytagel, 1 mg/l 2,4-D, and 0.1 mg/l BA, as described (Li et al. 2006). Twenty pieces of embryogenic calli were used per treatment. The initial fresh weight of the calli was taken and the calli were then placed in a sterile Petri dish containing a wet filter paper to maintain moisture. The Petri dishes were sealed and subjected to irradiation treatment as described above. The calli were then transferred to the same culture medium and maintained in dark at 25°C. After 3 weeks, the calli were weighed again and transferred to the regeneration medium, which was similar to the culture medium with a different phytohormone combination (1 mg/l BA, 0.2 mg/l NAA, and 0.5 mg/l GA). The cultures were maintained in a lighted chamber at 25°C with a 16-h photoperiod (140 μmol/m^2/s cool white fluorescent irradiance). After 4 weeks, the regenerated shoots were transferred to the rooting medium (hormone-free, half MS medium). Eight weeks later, regenerated plantlets with shoot length over 0.5 cm were counted. A total of 490 regenerated plantlets were transplanted to pots containing Metro-Mix-200 soil and kept in a lighted culture room until established, and then transferred to a greenhouse at 25 ± 5°C.

Irradiation mutagenesis of single node cuttings: For production of numerous mutants (first batch), 500 single node cuttings of 'Raleigh' were collected from NCSU Lake Wheeler Turfgrass Field Lab in January, 2005 and treated with 50 or 70 Gy gamma ray (250 cuttings per treatment). A second batch of ca. 2800 single node cuttings were collected and irradiated at 70 Gy in February 2006. Irradiated cuttings were planted into soil and maintained for 3 months in the greenhouse to determine survival rate and to identify mutants.

Selection for morphological mutants: Plants obtained from the first batch of irradiated cuttings (500 total), or regenerated from the treated calli (490 total) were first screened for freezing tolerance (Li et al. 2009a). One hundred and five surviving plants were transplanted into pots and grown in the greenhouse for 1–3 months. All putative mutants, as determined by morphological changes, were then transferred to a tray for further evaluations. Plants recovered from the second batch were directly subjected to screening for morphological mutations. Twelve mutants with consistent morphological changes and growth vigour were transplanted to the field and further characterized.

Morphological characterization of mutant plant lines: When sufficient plant materials were obtained for a mutant line, a field trial was conducted. Field trials of mutants G1, GF and 904AG1 were transplanted in summer of 2006, whereas mutants 106G1–106G4, and 904G1–904G4 were planted in summer of 2007. Due to a shortage of GB1 materials, measurements of this line were taken in the greenhouse. The field test employed randomized complete block design with three replicates. Measurements were taken 3–4 months later on ten randomly picked stolons from each plot. Measurements included blade length, width and sheath length from the shoot situated on the third node of each stolon, the length and thickness of the third internode and stolon length (the length from the shoot tip to the fourth node). The number of inflorescence per plot was also counted when the inflorescences were present.

Statistical analysis: ANOVA was carried out by using SAS software (ver. 9.1, SAS Institute 2003). When significant differences ($P = 0.05$) were observed, the least significant difference (LSD, Steel et al. 1996) test was performed to detect differences between treatments.

Results

Dosage effect on single node cuttings and calli

In a preliminary experiment, a wide range of irradiation dosages, 15, 45, 100, 200, 400, 800 and 1600 Gy, were tested on single node cuttings of 'Raleigh'. It was found that dosages greater than 100 Gy were lethal whereas dosage as low as 15 Gy did not have appreciable effect on plant growth. Subsequently, dosages of 0, 50, 60, 70, 80, 90 or 100 Gy were further investigated, and Probit analysis (Finney 1971) indicated that the LD_{50} and LD_{20} dosages were 48.5 and 72.6 Gy, respectively (Fig. 1).

Tolerance of calli to gamma ray irradiation seemed to be slightly higher. A reduction in callus growth was observed starting at 50 Gy, but calli still maintained appreciable growth after treatment at 200 Gy (Table 1). Callus regeneration was not affected with dosage up to 50 Gy. Over 300 regenerated plants were recovered from irradiation of 25 or 50 Gy, which were comparable with the non-treated control. However, only 39 plants were regenerated when calli were exposed to 100 Gy and no regeneration was observed after calli were treated at 200 Gy (Table 1). A total of 734 plants were regenerated from

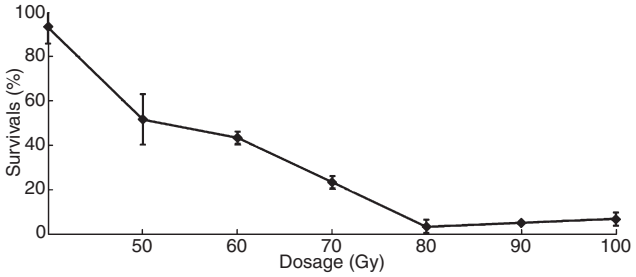

Fig. 1: Gamma ray dosage effects on survival of St. Augustinegrass cutting stolons (cv. 'Raleigh'). Each value represents means of three replicates with 20 single node cuttings per replicate. Bars represent SEs

Table 1: Effect of gamma ray dosage on callus growth and regeneration of cv. 'Raleigh'

Dosage (Gy)	Growth index $(W_1\text{-}W_0)/W_0$	Regenerated plant number per callus	Total plants regenerated
0	1.92	17	340
25	2.11	18	360
50	1.51	17	340
100	1.43	2	40
200	1.33	0	0

W_0, initial weight; W_1, the weight 3 weeks after irradiation.

the calli treated from 25 to 100 Gy, and 490 plants were selected based on their size and growth vigour. They were then transplanted to soil in the greenhouse for further investigation. Among them, three plant lines, 904G4, G1 and GB1, all regenerated from calli treated at 100 Gy, showed significant morphological changes, and were selected as mutant lines.

Irradiation mutagenesis with single node cuttings

Large scale irradiation of single node cuttings from January 2005 and February 2006 resulted in the selection of ten morphological mutants having a semi-dwarf growth trait. Seven of these, 106G1–G4, 904G1, GF and GF2, originated from 2005 irradiation events (50 or 70 Gy); and the remaining three lines (904AG1, 904G2, 904G3) were resulted from the 2006 treatment at 70 Gy.

Morphological characteristics of the mutants

As described above, a total of 13 morphological mutants were identified from the irradiated single node cuttings and calli. These lines performed consistently at different locations and in various years, suggesting that the observed altered traits were stable.

Mutant line G1, regenerated from 100 Gy-irradiated callus, had finer texture than 'Raleigh' in almost all the parameters measured. As shown in Table 2A and Figs 2 and 3, the leaf blades were narrower, and about 30% shorter than 'Raleigh'. The internodes were about 25% shorter, thinner and had shorter sheath lengths. In addition, compared with 'Raleigh', the culm length was reduced by 40%, the inflorescence shortened by 33% and height at maturation reduced by 25% (data not shown). On the contrary, G1 produced nearly eightfold as many inflorescences per unit area as 'Raleigh' did (Table 2A).

Line GF was recovered from single node cutting irradiated at 50 Gy. It was characterized by significantly shorter leaf blade lengths, shorter internodes and stolons were reduced by about 30% (Fig. 2 and Table 2A). GF also had fewer inflorescences per plot than 'Raleigh'. Line 904AG1 had significantly reduced lengths (by 20–30%) of leaf blades, internodes and stolons as shown in Fig. 2 and Table 2B. Line GF2 is another mutant recovered from single node cuttings at 50 Gy. It showed significantly shorter leaf length, leaf width, internode length, stolon length and sheath length (Table 2C). For example, the internode length was reduced by about 40%.

Lines 904G1–G4 and 106G1–G4 showed shortened internodes and stolons when growing in greenhouse at 25 ± 5°C (data not shown). After transplanting to the field, 904G1, 904G3, 904G4 and 106G4 either died or grew with low vigour. Lines 904G2, 106G1, 106G2, and 106G3 grew vigorously and maintained their mutant phenotypes. Lines 904G2, 106G1 and 106G2 showed more remarkable semi-dwarf growth. Their leaf lengths were shortened by 48%, 36%, and 22%, respectively,

Table 2: Morphological characteristics of the mutants

Genotype	LL (cm)	LW (cm)	ID (mm)	IL (cm)	SL (cm)	IN	SSL (cm)
A							
G1	1.31^{b}	0.40^{b}	2.43^{b}	3.73^{b}	14.66^{ab}	123.33^{a}	1.92^{c}
GF	1.28^{b}	0.45^{a}	2.91^{a}	3.22^{b}	13.01^{b}	0.67^{b}	2.47^{b}
Raleigh	1.93^{a}	0.46^{a}	3.05^{a}	4.88^{a}	18.53^{a}	16.33^{b}	3.02^{a}
CV%	13.94	14.94	5.19	6.74	4.87	15.16	9.34
B							
904AG1	1.73^{b}	0.44^{a}	2.75^{a}	3.50^{b}	11.94^{b}	–	–
Raleigh	2.32^{a}	0.47^{a}	3.35^{a}	5.31^{a}	14.39^{a}	–	–
CV%	6.37	5.59	6.23	16.68	10.66	–	–
C							
GF2	1.14^{b}	0.44^{b}	3.05^{a}	2.8^{b}	10.82^{b}	1.82^{b}	–
Raleigh	1.74^{a}	0.60^{a}	3.30^{a}	4.6^{a}	16.52^{a}	2.19^{a}	–
CV%	10.57	9.52	8.72	13.79	7.78	7.99	–
D							
106G1	1.07^{bc}	0.47^{b}	2.97^{ab}	1.5^{c}	7.0^{c}	–	1.67^{b}
106G2	1.30^{abc}	0.40^{b}	2.98^{ab}	1.8^{bc}	6.3^{c}	–	1.43^{b}
106G3	1.50^{ab}	0.47^{b}	2.94^{ab}	2.8^{b}	9.9^{b}	–	1.43^{b}
904G2	0.87^{c}	0.30^{b}	2.42^{b}	2.2^{bc}	8.0^{bc}	–	1.47^{b}
Raleigh	1.67^{a}	0.57^{a}	3.56^{a}	3.9^{a}	15.7^{a}	–	2.37^{a}
CV%	20.07	10.16	12.54	18.48	15.02	–	13.30

Each value represents means of three replicates. A and B: from two separate field trials of 2006 with 10 individual stolons measured from each replicate. C and D: from two separate field trials of 2007 with five individual stolons measured per replicate. Statistical analysis was performed among genotypes. Values followed by the same letter were not significantly different from each other according to the LSD ($P = 0.05$). CV, coefficient of variance; LL, leaf blade length; LW, leaf blade width; IL, internode length; ID, internode diameter; SL, stolon length; IN, inflorescence number per plot; SSL, sheath length.

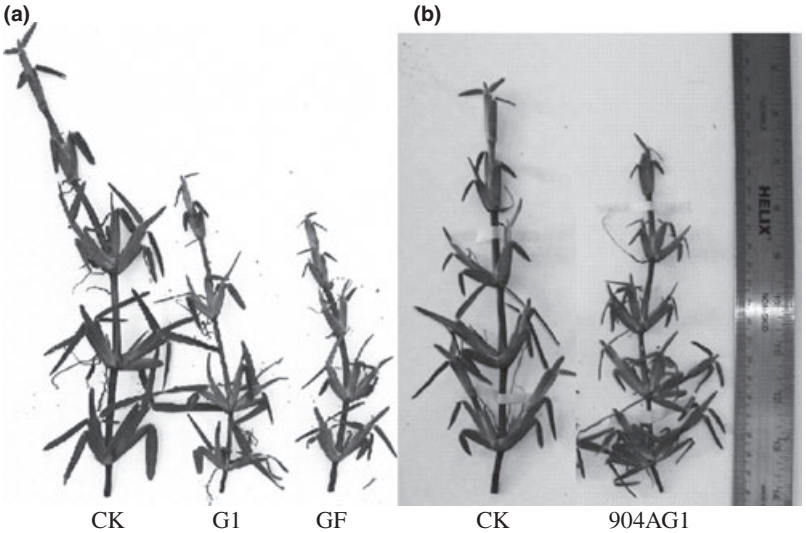

Fig. 2: Stolons with four internodes collected from mutants in the field trial at Lake Wheeler Turf Field Lab. (a) Mutants G1 and GF. (b) Mutant 904AG1. CK is 'Raleigh'

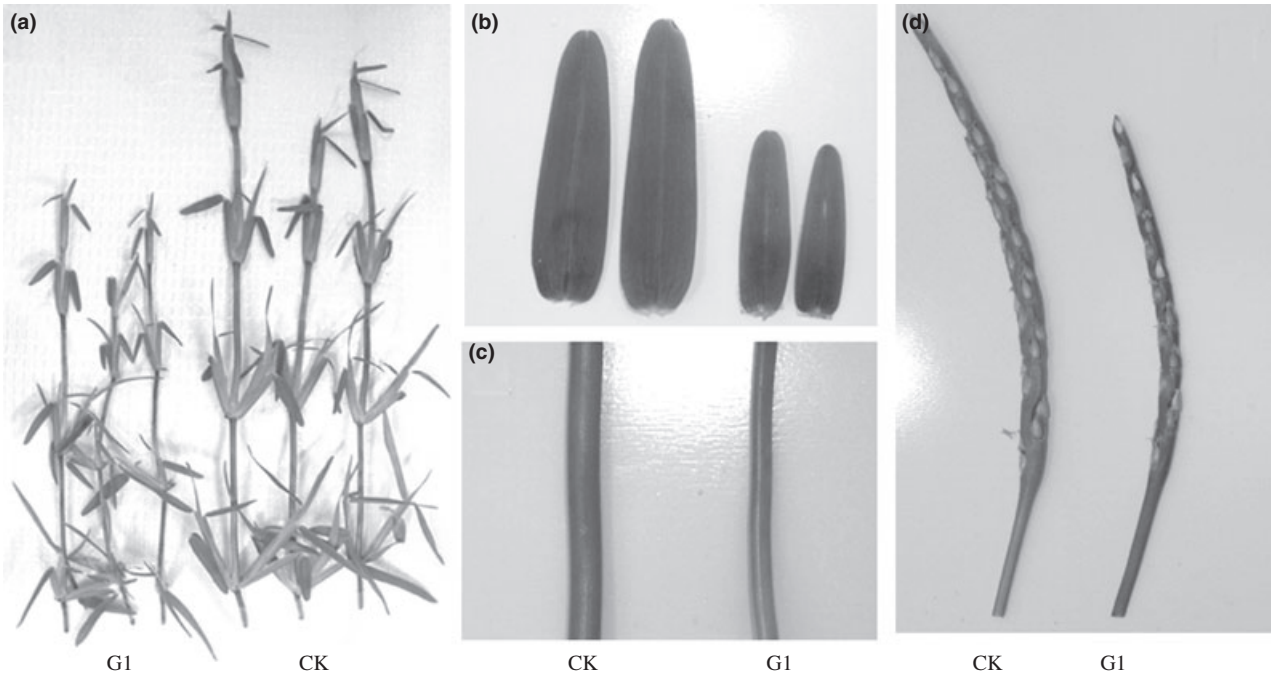

Fig. 3: Mutant G1 showed morphological changes at various tissues. (a) shorter stolons (b) finer leaf blades (c) thinner internode (d) shorter inflorescence. CK, 'Raleigh'

when compared with 'Raleigh'. Their internode lengths were reduced by 62%, 54% and 44%, respectively, and stolon lengths were decreased by 49%, 55% and 60% (Table 2D).

GB1 showed a different phenotype from other selected mutants. It was regenerated from callus irradiated with 100 Gy. GB1 grew vigorously but had a bunch-type growth in comparison with the horizontal spreading via stolons in wild type. After 6 months of growth, GB1 produced 80% fewer stolons that were 90% shorter than stolons measured for 'Raleigh' (Table 3).

GB2 did not establish well in the field and no data was collected. Evaluations thus far identified GF, GF2 and 106G3 as having superior field performance. Therefore, these lines have been included in multi-location and multi-year field trials.

Discussion

Our experiments demonstrate that both node cuttings from field-grown plants and calli from tissue culture are suitable for gamma ray irradiation mutagenesis in St. Augustinegrass. We identified 48.5 Gy as LD_{50} for node cuttings of 'Raleigh'. It is similar to 'Floratine', 'FA-243' and 'Floratam', but different from 'Bitterblue' (Busey 1980). Mutants were identified with significant morphological changes from the treated nodes and the changes were stable during vegetative propagation. Ten mutants were obtained from 3300 node cuttings treated at 50 or 70 Gy, giving a rate of 0.3%.

Table 3: Morphological characteristics of GB1 grown in a tray inside a greenhouse

Genotypes	No. of stolons spreading out of the tray	Stolon length (cm)	Internode length (cm)
GB1	5	13.4[b]	3.3[b]
Raleigh	25	123.8[a]	8.0[a]
CV%	–	22.72	5.76

Each value represents means measured from five stolons per genotype. Statistical analysis was performed between GB1 and 'Raleigh'. Values followed by the same letter were not significantly different from each other at the 5% level by LSD. Coefficient of variance is also presented.

There had been no study on mutagenesis using calli as starting materials for grass. In our experiments, St. Augustinegrass calli seemed to have more tolerance to irradiation treatment than single node cuttings. Regeneration ability was not affected when calli were treated at a dosage up to 50 Gy. However, when treated at 100 Gy, all the node cuttings were killed whereas calli still grew by 43% in weight and plants were still regenerated although at a reduced rate. Three mutants were recovered from the 39 plants regenerated from 20 pieces of calli treated at 100 Gy whereas no mutant was identified from treatments at lower dosages. Although the number of the calli treated at this dosage is too small to estimate the mutagenesis rate, the results did indicate that the approach and the conditions may be a more efficient way to perform plant mutagenesis, and is worth of future investigations in other grass species. Although the chance is low, we cannot completely exclude the possibility that the mutants we observed from the regenerated plants were somaclonal variants induced by tissue culture rather than the results of gamma ray irradiation. However, in our other experiments dealing with somaclonal variation, we observed that the frequency of somaclonal variation in St. Augustinegrass was around 0.2% (Li et al. 2009b). Moreover, the fact that no mutant was recovered from nearly 700 plants regenerated from calli treated at 25 or 50 Gy supported the note that what we isolated were mutants rather than somaclonal variants.

Probably because we were looking for finer plant texture, and morphological mutants that are easier to identify, all the mutants we isolated were semi-dwarf type with significantly shortened internodes and stolons. The compact growth pattern and finer leaves improve the turf quality (Reynolds et al. 2009). In addition, the semi-dwarf type mutants seem to have denser root systems which could provide harvesting advanta-

ges for sod farmers. Semi-dwarf mutants of St. Augustinegrass were observed by Powell and Toler (1980) from irradiated stolons of Floratam. However, most of the mutants reportedly grew weakly with some incapable of surviving at field conditions (Reinert et al. 1981). In contrast to that observation, a majority of the semi-dwarf mutants we discovered grew with good vigour, and established well in the field. These mutants would enlarge the germplasm pool of St. Augustinegrass and serve as good breeding materials. As they were derived from 'Raleigh', they bear a good cold tolerance genetic background. Field tests of the mutant lines in the past 2 years seemed to confirm that they retained this favourable trait (Reynolds et al. 2009). Besides freezing tolerance, St. Augustine decline and southern chinch bugs are serious problems in St. Augustinegrass management (Busey 2003). Thus, evaluation of resistance of the mutants to these pests would be a necessary step during cultivar development (Reinert et al. 1981).

Dwarf or semi-dwarf mutants have been isolated in many plant species and have been extensively analysed for their mechanisms and, particularly, responses to plant hormones (Murfet and Reid 1997, Kwon and Choe 2005). Various dwarf phenotypes have been associated with defection in biosynthesis or reception of gibberellins (Ross et al. 1997), brassinosteroids (Noguchi et al. 1999), regulation of cell elongation (Takahashi et al. 1995) or with abnormal cell walls (Reiter et al. 1993). Among the semi-dwarf mutants we isolated, G1 is particularly interesting since it also has much more inflorescences. Although the molecular basis of this phenotype is not understood, a similar case found in rice may provide insights of the mechanisms. Rice mutant htd-1 is dwarf and has an increased number of tillers (Zou et al. 2005). It turned out that the HTD1 gene is an orthologue of Arabidopsis MAX3, which encodes a carotenoid cleavage dioxygenase involved in the synthesis of a carotenoid-derived signal molecule. High tillering is a result of release of the axillary buds and the excessive tiller growth may result in the dwarf phenotype (Zou et al. 2006). It would be interesting to further characterize the mutants we isolated to gain insights of the mechanisms behind the altered phenotypes, and explain why most of the mutants have semi-dwarf growth habit.

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