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Short Daylength Response in Mutant Soybean Plants at Vegetative Growth Stage

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Abstract

In this study mutant (M3 and M4) soybean lines were selected and examined in two daylength conditions. The mutant populations were developed from Chinese wild type ZP661 since 0.62% EMS mutagen was added. The study comprises 20 M3 and M4 soybean lines assessed for their sensitivity to short day condition expressed in seed germination, root length, fresh weight and plant height at vegetative growth stage and flowering time. Two genotypes PI196160 and W0001225 were used as sensitive and less-sensitive control. Short daylength with 16 and 8 hours as dark and light respectively and standard daylength conditions with 12 hours fixed in light and dark, were instructed in growth chambers. Factorial Analysis of data collected from number of germinated plants revealed differences in the two daylength conditions and their interaction. The line 3ms 097-3 showed less-sensitivity with 100% germination in the two days conditions. A significant difference was observed in plant height at different time of growing period. Differences in root length were observed in both conditions at two weeks after planting. The result of the experiment showed line 3ms 097-3 as less-sensitive to short day expressed in plant height, root length (12.7cm) and fresh weight (2.9gm). This line could be advanced for further testing in the field conditions before used as source of less-sensitive to short daylength in future breeding program.

Keywords: Soybean; Short day response; Germination; Plant height; Root

Introduction

Becoming less-sensitive to short daylength plant means is to have a system developed irresponsive to a day difference. In theory plants with such mechanisms will perform well in short day conditions that might lead to break the seasonality of soybean production. The succession of short and long daylength as a result of earth rotation lead plants to develop mechanism responding to environmental cues known as circadian clock which is the endogenous mechanism constructed to be respondent to daylength and gives plants the capability to predict these changes and modify their developmental programs to improve plant adaptation level to those environmental hints. Physiological studies of delaying in flowering on mutant and different species revealed that flowering is disturb by multiple environmental factors, such as daylength, heat level and light quality [1-5]. Long-day conditions boost internode length, leaf area, and dry weight and shortening the time for formation of successive leaf pairs in S. rebaudiana as compared with short days [6]. In strawberry daylength also regulate vegetative growth of axillary buds [7]. Another, studies identified a chlorophyll-deficient mutant that lead to death [8]. In rice it's suggested that development of adapted plants to northern areas is required reducing their sensitivity to photoperiod, by capacitate plants to flower normally under longer daylength conditions before the onset of winter [9]. Modifications of the plant photoperiodic network by enforced selection have been instrumental for this purpose. Chemical mutagens were intensively used to obtain mutations in soybean populations that were found to contain a variety of plant type traits, create variability in fatty acid composition, and meal quality traits [10-14].

The screening of soybean for seed germination, root length, fresh weight and plant height will lead to better grasp of knowledge of ecological range and potential for selection of new lines adapted to wide range of environments. One study was carried out to determine the impact of light intensity in unfavorable cold conditions in two soybean cultivars. The result of the soybean plants under cold stress and different light intensities, showed that soybean plant grown in cold stress conditions at lower light intensity

were less affected than those plants grown in normal light conditions [15]. Khudhair et al. [16] exposed soybean seeds to radiation of 0, 100, 200, and 300kR of gamma rays, through his work two mutants showed as superior in their yield components and earliness compared with the H226 and other control cultivars. The work aimed to provide breeding programs with new lines neutral to daylength conditions. The specific objectives to study the effect of short day on germination, plant vigorous expressed in plant height, root length and fresh weight of mutant soybean at vegetative growth stage and at flowering time.

Materials and Methods

Development of mutant populations

The Chinese wild soybean ZP661 seeds were neutralized by 10% sodium thiosulfate solution and then seeds were soaked in 0.62% EMS for 8 hours. The treated soybean seeds were water washed before planting. Individual M1, a total of 21,600 M2 plants were planted and observed phenotypically (Figure 1). Then 20 M3 and M4 derived progenies were selected and tested in growth chamber conditions (Figure 1). Two wild types P1196160 and W0001265 were used as sensitive and les-sensitive control to short day cues, respectively.

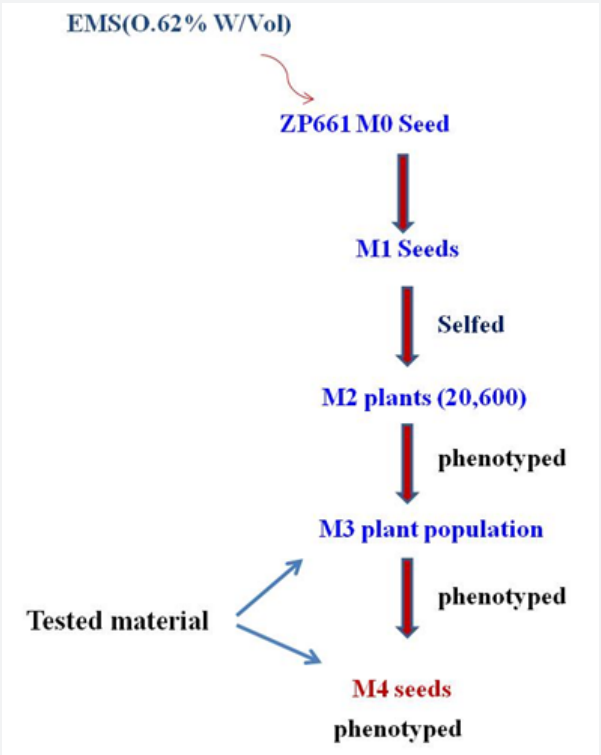

Figure 1: Developmental scheme of plant materials.

Short and standard daylength conditions

The experiment was conducted in two diurnal conditions; one set constitute 20 M3 and M4 plants grown in short day condition using 16 and eight hours as dark and light respectively. However, another set of 20 M3 and M4 plants grown in standard day condition using 12h for dark and 12h for light. Two plant control. The temperature was adjusted at 20°C and 28°C as night and day time temperature, respectively for both conditions.

Data collection and statistical analysis: Factorial experiment with three replicates complete randomized (CRD) was designed. The phenotypic data was performed according to IBPGR (1984) with some modifications. The plants were observed for germination for four days started from the third day after planting. The plant height was measured every 24 hours for the first two weeks after plant emergence, 30 and 45 days after germination and at flowering. While the root length and fresh weight measured once from destructive sample carried out after two weeks since planting. The Analysis of variance (ANOVA) of number of plants germinated, root length, fresh weight and plant height was performed using SAS to obtain P value, level of superiority and grand mean. GenStat Discovery edition 3 [17] was also used to obtain clusters.

Results and Discussions

EMS, one of the damaging alkylating agents used intensively to induce point mutation in plant species. Induced mutagenesis is extensively used to improve crop varieties or create new genetic variations. In this study 0.62% EMS used to develop mutant populations. While Cooper et al. [18] used 0.4 and 0.5% EMS to develop soybean mutant populations. In the chemical was also used in Arabidopsis with 20mM to 30mM [19]. The use of mutation in Korean soybean was led to two seed-coat color mutant cultivars were developed through g-irradiation at a dose of 250Gy [20].

Plant germination

Its known and proved that short daylength condition is the amount of darkness that determined when the plants flower and how many flowers were produced. Some plants established better when the darkness hours were extended, these plants are described as short-day plants. However, other plants that performed better with fewer hours of darkness are described as long-day plants. Further research identified that there were plants that stable in respond to long periods of darkness, these plants are described as day-neutral plants.

In this respect, the number of germinated plants was used to measure plant reaction to short day condition. Investigators emphasized that temperature, light, pH, and soil moisture are disturbing seed germination [21,22]. Seed vigor influenced field emergence, speed of germination and initial plant size [23]. The plant germination was carried out at the day fourth after plant emergence. The factorial analysis revealed significant difference of number of plants germinated in genotypes, the two conditions and interaction between the genotypes and the two conditions (Table 1). Analysis of data of number of emerged plats showed differenc-

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es in the degree of sensitivity or tolerance to short daylength condition. The result of number of emerged plants showed line 3ms 097-3 with 100% germination in short day condition after five days of seeding. This result is similar to the less-sensitive control W0001265, and significantly different from the sensitive control PI196160 (Table 1). We consider the line 3ms 097-3 as stable in the two conditions, since the reaction is similar to W0001265 and compared to PI196160, the two controls used as less responsive to long and short daylength cues respectively (Table 1). Chemical mutagens have been extensively used for many phenotypic screens in soybean, providing lines with new phenotypes such as ethylene sensitivity and nodulation [24,25]. The line 3ms 113-6, 4ms 147-5 and 4ms 148-4 not germinated even after six days since seeding at short day condition. We consider these lines are responsive to short daylength cues expressed in number of plants germinated (Table 1). Mutations are useful with very low frequencies, while the treatment with damaging mutagens make considerable effect by reducing plant emergence, growth speed, vigor and pollen and ovule fertility in the living organisms [26,27].

Table 1: Germinated plants in Standard and short-day conditions.

Lines Information		STD Condition				SD Condition			
Designation	Genotype	Day 3	Day 4	Day 5	Day 6	Day 3	Day 4	Day 5	Day 6
qiu 1	M3	1.00^{de}	2.00^{c}	2.00^{c}	2.00^{dc}	0.00^{d}	1.00^{ed}	1.00^{ed}	1.00^{de}
qiu 2	M3	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	1.00^{c}	2.00^{d}	3.00^{cb}	3.00^{bc}
3ms-089-7	M3	3.00^{bc}	5.00^{a}	5.00^{a}	5.00^{a}	0.00^{d}	2.00^{d}	2.00^{cd}	2.00^{dc}
3ms-095-6	M3	3.00^{bc}	3	4.00b^{a}	4.00^{ba}	1.00^{c}	1.00^{ed}	3.00^{cb}	3.00^{bc}
3ms-097-3	M3	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	3.00^{a}	4.00^{b}	5.00^{a}	5.00^{a}
3ms-102-5	M3	3.00^{bc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	1.00^{c}	1.00^{ed}	1.00^{ed}	1.00^{de}
3ms-104-6	M3	1.00^{de}	2.00^{c}	2.00^{c}	2.00^{dc}	0.00^{d}	1.00^{ed}	1.00^{ed}	1.00^{de}
3ms-113-6	M4	3.00^{bc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}
4ms-146-1	M4	3.00^{bc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	2.00^{b}	2.00^{d}	3.00^{cb}	3.00^{bc}
4ms-147-5	M4	3.00^{bc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	0.00 ^{d}	0.00^{e}	0.00^{e}	0.00^{e}
4ms-148-4	M4	3.00^{bc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	0.00 ^{d}	0.00^{e}	0.00^{e}	0.00^{e}
4ms-156-2a	M4	1.00^{de}	2.00^{c}	2.00^{c}	3.00^{bc}	3.00^{a}	3.00^{c}	3.00^{cb}	3.00^{bc}
4ms-156-5	M4	2.00^{dc}	2.00^{c}	2.00^{c}	2.00^{dc}	3.00^{a}	2.00^{d}	4.00^{b}	4.00^{ba}
4ms-161-10	M4	2.00^{dc}	3.00^{bc}	3.00b^{c}	3.00^{bc}	2.00^{b}	2.00^{d}	4.00^{b}	4.00^{ba}
4ms-161-3	M4	4.00^{ba}	5.00^{a}	5.00^{a}	5.00^{a}	0.00^{d}	0.00^{e}	2.00^{cd}	3.00^{bc}
4ms-168-1	M4	3.00^{bc}	5.00^{a}	5.00^{a}	5.00^{a}	0.00^{d}	0.00^{e}	1.00^{ed}	3.00^{bc}
4ms-183-9	M4	2.00^{dc}	2.00^{c}	2.00^{c}	2.00^{dc}	3.00^{a}	3.00^{c}	3.00^{cb}	5.00^{a}
4ms-184-4	M4	0.00^{e}	0.00^{d}	0.00^{d}	1.00^{d}	3.00^{a}	4.00^{b}	4.00^{b}	4.00^{ba}
4ms-189-5	M4	0.00^{e}	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{e}	2.00^{dc}
4ms-295-9	M4	3.00^{bc}	4.00b^{a}	4.00^{ba}	4.00^{ba}	1.00^{c}	1.00^{ed}	1.00^{ed}	1.00^{de}
W0001265	control1	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	2.00^{b}	5.00^{a}	5.00^{a}	5.00^{a}
PI196160	control2	3.00^{bc}	5.00^{a}	5.00^{a}	5.00^{a}	1.00^{c}	3.00^{c}	3.00^{cb}	3.00^{bc}
Mean		2.64	3.18	3.23	3.32	1.19	1.69	2.23	2.55
P		<.000	<.000	<.000	<.000	<.000	<.000	<.000	<.000

Means's with different letters in the same column are significantly different at P = 0.05

Plant height

Plant vigorous can be defined as plants grow bigger or exhibit greater biomass, growth rate and development [28]. In this context, we use every day change in plant height at early growth stage particularly in the first two weeks as plant vigorous indicator to short daylength condition.

Factorial analysis of variance of height of plants grown in two adjusted daylength conditions, a significant difference in the consecutive 12 days after plant emerged was observed in all treatments and the two days conditions and their interaction. The tested plants varied in their sensitivity to the two daylength conditions. In this experiment soybean plants grown in short daylength were observed as shorter than the ones grown in standard day condition (Table 1 & 2). The 3m097-3 showed stability in both conditions when compared to the line W0001265 the les-sensitive control. In previous studies, a plant considered as a long daylength or a short daylength respondent species is précised by exposure period to daylight or darkness needed by plants to grow, flower or

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change color. According to that, plants were classified into three groups short day (SD), Long day (LD) and Neutral (ND) [29]. In other study, some cultivars were showed sensitivity to short day conditions since complete initiation of cotyledon [30].

Table 2: Germinated plants in Standard and short-day conditions.

Designation	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day15
qiu 1	0.00^{c}	0.06^{d}	0.20^{c}	0.20^{fc}	1.56^{dc}	2.30^{edc}	2.70^{edf}	3.14^{ef}	3.98^{ef}	4.70^{edf}	6.30^{ed}	7.60^{edf}	8.42^{edcf}
qiu 2	0. 06^{bc}	2.00^{a}	3.28^{a}	5.84^{ba}	7.90^{ba}	10.60^{ba}	12.60^{ba}	14.56^{ba}	17.26^{bac}	19.62^{ba}	21.52^{ba}	23.06^{bac}	23.76^{bac}
3ms-089-7	0.00^{c}	0.16^{d}	0.50^{c}	1.90^{edfc}	3.08^{bdc}	3.60^{edc}	4.56^{ebdcf}	5.14^{ebdcf}	6.16^{edcf}	6.76^{edcf}	7.68^{ebdc}	8.44^{edcf}	9.12^{edcf}
3ms-095-6	0. 06^{bc}	0.10^{d}	0.28^{c}	0.80^{edfc}	2.72^{bdc}	3.82^{ebdc}	5.90^{ebdcf}	7.74^{ebdcf}	10.80$^{eb-dacf}$	12.28$^{eb-dacf}$	15.50^{bdac}	18.60^{eb-dac}	20.48^{bdac}
3ms-097-3	0.30^{ba}	1.40^{ba}	3.20^{a}	6.88^{a}	9.82^{a}	13.28^{a}	16.26^{a}	18.14^{a}	20.50^{a}	21.80^{a}	24.68^{a}	25.70^{a}	27.14^{a}
3ms-102-5	0.06^{bc}	0.30^{cd}	0.65^{c}	1.04^{edfc}	1.90^{dc}	2.60^{edc}	3.60^{edcf}	4.70^{edcf}	6.24^{edcf}	7.20^{ebdcf}	8.00^{ebdc}	9.00^{edcf}	9.74^{ebdcf}
3ms-104-6	0.00^{c}	0.10^{d}	0.20^{c}	0.50^{edf}	1.10^{dc}	1.70^{ed}	2.10^{ef}	2.44^{f}	3.20^{ef}	3.36^{ef}	4.00^{ed}	4.50^{ef}	4.80^{ef}
3ms-113-6	0.00^{c}	0.00^{d}	0.00^{c}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{f}
4ms-146-1	0.26^{bac}	0.80^{bcd}	1.76b^{ac}	2.94^{ebdfc}	4.00^{bdc}	4.90^{ebdc}	5.64^{ebdcf}	6.36^{ebdcf}	7.50^{ebdcf}	8.26^{ebdcf}	9.38^{ebdc}	10.80^{eb-dcf}	11.82$^{eb-dacf}$
4ms-147-5	0.00^{c}	0.00^{d}	0.00^{c}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{f}
4ms-148-4	0.00^{c}	0.00^{d}	0.00^{c}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{f}
4ms-156-2a	0.14^{bac}	1.12^{bc}	1.98^{bac}	4.02^{bdac}	5.80^{bac}	7.12^{bdac}	8.20^{ebdacf}	9.02^{ebdacf}	10.40$^{eb-dacf}$	11.14$^{eb-dacf}$	13.24^{eb-dac}	13.66$^{eb-dacf}$	14.42$^{eb-dacf}$
4ms-156-5	0.32^{a}	0.86^{bcd}	1.82^{bac}	3.30^{ebdfc}	5.36^{bdac}	7.44^{bdac}	8.86^{ebdac}	9.90^{ebdacf}	11.60$^{eb-dacf}$	12.44$^{eb-dacf}$	13.68^{eb-dac}	14.50$^{eb-dacf}$	15.10$^{eb-dacf}$
4ms-161-10	0.20^{bac}	0.50^{cd}	1.02^{bc}	2.80^{ebdfc}	4.94^{bdac}	7.60^{bdac}	11.06^{bdac}	13.24^{bdac}	16.40^{bdac}	17.34^{bdac}	20.20^{bac}	22.00^{bdac}	23.14^{bac}
4ms-161-3	0.00^{c}	0.00^{d}	0.14^{c}	1.20^{edfc}	2.22^{dc}	3.30^{edc}	4.60^{ebdcf}	6.50^{ebdcf}	7.40^{ebdcf}	9.16^{ebdcf}	10.02^{ebdc}	11.70$^{eb-dacf}$	12.54$^{eb-dacf}$
4ms-168-1	0.00^{c}	0.12^{d}	0.90^{bc}	1.80^{edfc}	2.78^{bdc}	3.90^{ebdc}	4.80^{ebdcf}	5.74^{ebdcf}	6.60^{edcf}	7.02^{ebdcf}	7.80^{ebdc}	8.86^{edcf}	9.70^{ebdcf}
4ms-183-9	0.26^{bac}	0.92^{bcd}	1.64^{bac}	3.84^{ebdac}	5.72^{bac}	8.50^{bdac}	10.50^{eb-dac}	12.30^{eb-dac}	14.30^{eb-dac}	15.78^{eb-dac}	17.36^{bdac}	18.90^{eb-dac}	20.12^{ebdac}
4ms-184-4	0.24^{bac}	1.48^{ba}	2.74^{ba}	4.40^{bac}	5.96^{bac}	7.46^{bda}	8.54^{ebdacf}	9.00^{ebdacf}	10.50$^{eb-dacf}$	11.16$^{eb-dacf}$	12.16^{eb-dac}	12.82$^{eb-dacf}$	13.12$^{eb-dacf}$
4ms-189-5	0.00^{c}	0.00^{d}	0.00^{c}	0.50^{edf}	1.28^{dc}	2.06^{edc}	2.76^{edf}	3.40^{ef}	4.84^{edf}	5.46^{edf}	7.46^{edc}	8.30^{edcf}	8.88^{edcf}
4ms-295-9	0. 04^{c}	0.26^{cd}	0.54^{c}	1.20^{edfc}	2.08^{dc}	2.60^{edc}	3.50^{edcf}	4.18^{edf}	5.08^{edf}	5.52^{edf}	5.98^{ed}	6.60^{ef}	6.88^{edf}
W000 1265	0.10^{bac}	0.54^{cd}	1.20^{bc}	3.34^{ebdfc}	6.05^{bac}	8.90^{bac}	11.74^{bac}	14.40^{bac}	18.40^{ba}	19.30^{bac}	21.30^{bac}	23.68^{ba}	24.96^{ba}
PI1 96160	0.06^{bc}	0.26^{cd}	1.00^{bc}	2.70^{ebdfc}	4.30^{bdc}	6.80^{ebdac}	8.30^{ebdacf}	9.05^{ebdacf}	11.40$^{eb-dacf}$	11.74$^{eb-dacf}$	12.86^{eb-dac}	14.20$^{eb-dacf}$	15.36$^{eb-dacf}$
Mean	0.1	0.5	1.04	2.24	3.57	4.9	6.19	7.22	8.75	9.54	10.83	11.95	12.7
P <	<0.005	<0.000	<0.000	<0.001	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000

Means's with different letters in the same column are significantly different at P = 0.05

Photoperiodism is the one of reason that soybean plants cultivated in summer season at different areas of the crop make limitations for crop to be cultivated in season with short day condition. Investigators suggested that the duration as factor affecting

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plants rather than the amount of light as a circumstances disturbing plant development [31-33]. Plant with neutral behavior is encouraged and will have impact in breaking the seasonality of crop production. The result showed the line 3ms097-3 as less-sensitive through the small change showed in plant height in both day length conditions.

Table 3: Plant height of plants grown in standard day condition during the first two weeks of growing.

Designation	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
qiu 1	0.16^{bdc}	0.76^{dc}	2.10^{ebdc}	3.60^{ebdc}	4.60^{ebdc}	5.40^{bdc}	6.00^{ebdcf}	6.76^{ebdc}	8.06^{edgcf}	9.18^{ebdfc}	10.64^{bdec}	11.80^{ebdfc}	12.74$^{eb-dgcf}$
qiu 2	0.96^{bac}	1.92^{bac}	5.90^{ba}	9.86^{a}	12.04^{a}	14.80^{a}	16.40^{a}	18.86^{a}	22.44^{ba}	26.00^{a}	29.00^{a}	30.70^{a}	33.52^{a}
3ms-089-7	1. 02^{ba}	2.90^{a}	5.56b^{ac}	7.60^{bdac}	8.66^{bac}	10.06^{ba}	11.72^{bdac}	13.24^{bac}	15.68^{eb-dac}	17.40^{bdac}	18.80^{bdac}	20.20^{eb-dac}	21.94$^{eb-dacf}$
3ms-095-6	0.26^{bdc}	0.80^{dc}	3.40^{ebdac}	5.40^{ebdac}	7.44^{bac}	9.50^{ba}	11.46^{bdac}	13.50^{bac}	15.94^{eb-dac}	18.50^{bdac}	21.24^{bac}	22.50^{bdac}	25.08^{eb-dac}
3ms-097-3	1.02 ^{ba}	2.04^{bac}	6.80^{a}	9.32^{ba}	12.76^{a}	15.50^{a}	17.20^{a}	19.36^{a}	22.88^{a}	25.52^{a}	28.62^{a}	30.60^{a}	32.88^{a}
3ms-102-5	0.84^{bdac}	1.96^{bac}	4.96^{bdac}	5.90^{bdac}	6.84^{bdac}	8.28^{bac}	9.4^{ebdac}	10.00^{eb-dac}	13.94$^{eb-dacf}$	15.24^{eb-dac}	17.00^{bdac}	18.80^{eb-dac}	20.84$^{eb-dacf}$
3ms-104-6	0.10^{dc}	0.40^{dc}	1.00^{ed}	2.34^{ed}	2.98^{edc}	4.00^{bdc}	5.10^{edcf}	5.90^{ebdc}	7.00^{edgf}	7.50^{edfc}	8.22^{dec}	9.00^{edf}	9.70^{edgf}
3ms-113-6	0.38^{bdc}	1.36^{bdac}	2.88^{ebdc}	4.58^{ebdac}	6.68^{ebdac}	8.66^{bac}	9.80^{ebdac}	11.10^{bdac}	13.00$^{eb-dacf}$	16.32^{eb-dac}	17.88^{bdac}	19.40^{eb-dac}	20.56$^{eb-dacf}$
4ms-146-1	0.84^{bdac}	2.24^{bac}	4.10^{bdac}	6.74^{bdac}	7.58^{bac}	10.54^{ba}	11.50^{bdac}	12.36^{bac}	15.40^{eb-dac}	17.40^{bdac}	18.60^{bdac}	20.30^{eb-dac}	21.84$^{eb-dacf}$
4ms-147-5	0.24^{bdc}	1.46^{bdac}	2.56^{ebdc}	4.20^{ebdac}	6.46^{ebdac}	8.70^{bac}	10.64^{bdac}	12.66^{bac}	14.76^{eb-dac}	16.28^{eb-dac}	17.20^{bdac}	18.50^{eb-dac}	19.90$^{eb-dacf}$
4ms-148-4	0.22^{bdc}	0.92^{bdc}	2.42^{ebdc}	4.70^{ebdac}	7.84^{bac}	9.60^{ba}	11.60^{bdac}	14.24^{bac}	16.06^{eb-dac}	17.72^{bdac}	19.10^{bdac}	20.24^{eb-dac}	21.44$^{eb-dacf}$
4ms-156-2a	0.14^{bdc}	0.80^{dc}	1.80^{edc}	3.00^{edc}	3.90^{ebdc}	6.14^{bdc}	8.06^{ebdacf}	9.64^{ebdac}	11.90$^{eb-dgacf}$	14.08^{eb-dac}	16.10^{bdac}	17.80^{eb-dac}	19.68$^{eb-dacf}$
4ms-156-5	0.64^{bdac}	0.86^{bdc}	1.74^{edc}	3.10^{edc}	3.74^{ebdc}	4.38^{bdc}	5.20^{edcf}	6.10^{ebdc}	7.26^{edgf}	7.80^{edfc}	8.90^{bdec}	9.60^{edfc}	10.36^{edgcf}
4ms-161-10	0.26^{bdc}	1.34^{bdac}	2.70^{ebdc}	6.76^{bdac}	8.04^{bac}	11.20^{ba}	13.28^{bdac}	15.46^{bac}	18.14^{eb-dac}	20.80^{bdac}	24.22^{ba}	26.70^{bac}	29.28^{ba}
4ms-161-3	0.92^{bac}	2.16^{bac}	4.12^{bdac}	6.04^{bdac}	8.68^{bac}	11.36^{ba}	12.40^{bdac}	14.70^{bac}	17.84^{eb-dac}	20.52^{bdac}	23.00^{bac}	24.76^{bdac}	27.16^{bdac}
4ms-168-1	0.96^{bac}	1.80^{bdac}	3.80^{ebdac}	4.50^{ebdac}	6.30^{ebdac}	7.28^{bdac}	7.96^{ebdacf}	8.66^{ebdac}	9.70^{ebdgcf}	10.20^{ebdfc}	11.24^{bdec}	12.40^{ebdfc}	13.46$^{eb-dgcf}$
4ms-183-9	0.50^{bdc}	1.3^{bdac}	1.83^{ebdc}	3.40^{edc}	3.70^{ebdc}	4.10^{bdc}	4.46^{edf}	4.76^{ebdc}	5.70^{egf}	6.46^{edf}	7.66^{dec}	8.10^{edf}	8.34^{egf}
4ms-184-4	0.00^{d}	0.00^{d}	0.00^{e}	0.20^{e}	0.50^{ed}	0.80^{dc}	0. 80^{ef}	1.08^{ed}	2.16^{gf}	3.83^{ef}	4.50^{de}	5.00^{ef}	5.80^{gf}
4ms-189-5	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{d}	0.00^{f}	0.00^{e}	0.00^{g}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{g}
4ms-295-9	0.42^{bdc}	1.86^{bdac}	3.80^{ebdac}	6.80^{bdac}	8.58^{bac}	10.62^{ba}	12.10^{bdac}	14.26^{bac}	17.18^{eb-dac}	18.48^{bdac}	20.96^{bac}	23.10^{bdac}	25.10^{eb-dac}
W000 1265	1.40^{a}	2.70^{ba}	5.40^{bac}	8.40^{bac}	10.04^{ba}	12.46^{ba}	14.80^{ba}	17.86^{a}	20.74^{bac}	22.74^{ba}	24.80^{ba}	27.20^{ba}	28.50^{bac}
PI1 96160	0.42^{bdc}	1.66^{bdac}	4.86^{bdac}	7.90^{bdac}	9.90^{bac}	12.28^{ba}	14.10^{bac}	15.96^{ba}	19.40^{bdac}	21.68^{bac}	24.50^{ba}	27.40^{ba}	30.06^{ba}
Mean	0.53	1.42	3.45	5.2	6.69	8.43	9.72	11.25	13.42	15.13	16.92	18.37	19.91
P	0.002	0.01	0.002	0.002	<.0007	<.000	<.001	<.000	<.000	<.000	<.000	<.000	<.000

Means's with different letters in the same column are significantly different at P = 0.05

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The plant height result of 30 and 45 days since planting showed plants grown in short day as shorter than plants grown in standard day condition (Table 3). The obtained results suggested that difference in results on plant respond to short day condition.

The plant height result at flowering time revealed that plants grown in short daylength as shorter than plants grown in standard day condition (Table 4). The increment rate in plant from 45 days to flowering time observed as the shortest difference through plant growth stage. It is probable that their rate of increase would have decreased when the rate of growth decreased to allow plants begin flowering. In order to overcome the complexity associated with growing of plant species, growth analysis is providing effective method to track progress and measure the involvement level of different physiological processes on the plant behavior [34].

Table 4: Plant height of plants grown in standard day condition after 30, 45 days and flowerings.

Designation	STD Condition			SD Condition		
	Day 30	Day 45	Flowering	Day 30	Day 45	Flowering
qiu 1	17.62^{bdc}	24.4^{bdc}	24.90^{bdc}	23.60^{ebdac}	28.60^{bdac}	32.10^{bdac}
qiu 2	40.04^{ba}	52.72^{bac}	57.20^{ba}	28.54^{bdac}	32.90^{bac}	40.5^{ba}
3ms-089-7	37.60^{bac}	45.40^{bac}	57.90^{ba}	9.60^{edf}	9.60^{ed}	9.70^{ed}
3ms-095-6	33.14^{bac}	48.20^{bac}	51.66^{bac}	35.88^{ba}	37.90^{ba}	39.30^{ba}
3ms-097-3	39.34^{bac}	46.50^{bac}	47.70^{bac}	37.80^{a}	41.53^{a}	43.50^{a}
3ms-102-5	31.40^{bac}	35.70^{bac}	36.42^{bdac}	14.60^{ebdcf}	15.20^{ebdc}	15.80^{ebdc}
3ms-104-6	15.40^{bdc}	18.5^{dc}	18.70^{dc}	7.70^{ebdc}	9.00^{edc}	9.30^{edc}
3ms-113-6	30.20^{bac}	36.54^{bac}	37.80^{bac}	0.00^{f}	0.00^{e}	0.00^{e}
4ms-146-1	21.26^{bdac}	21.30^{bdc}	21.90^{bdc}	20.30^{ebdacf}	23.70^{ebdac}	24.00^{ebdac}
4ms-147-5	22.16^{bdac}	35.30^{bdac}	35.60^{bdac}	3.80^{ef}	6.00^{ed}	6.40^{ed}
4ms-148-4	34.20^{bac}	39.40^{bac}	47.66^{bac}	0.00^{f}	0.00^{e}	0.00^{e}
4ms-156-2^{a}	40.20^{bac}	41.40^{bac}	42.36^{bac}	28.90^{bdac}	37.70^{ba}	38.20^{ba}
4ms-156-5	18.50^{bdc}	23.70^{bdc}	27.30^{bdc}	20.30^{ebdacf}	24.50^{ebdac}	25.00^{ebdac}
4ms-161-10	49.94^{a}	65.58^{a}	73.26^{a}	32.00^{bac}	35.40^{ba}	37.20^{ba}
4ms-161-3	45.40^{ba}	54.20^{bac}	57.24^{ba}	24.40^{ebdac}	30.60^{bdac}	35.60^{bac}
4ms-168-1	27.60^{bdac}	34.90^{bdac}	36.64^{bdac}	19.20^{ebdacf}	28.00^{bdac}	28.40^{bdac}
4ms-183-9	14.80^{dc}	17.60^{dc}	18.20^{dc}	32.80^{bac}	37.60^{ba}	38.40^{ba}
4ms-184-4	17.10^{bdc}	21.50^{bdc}	25.00^{bdc}	19.80^{ebdacf}	24.40^{ebdac}	28.70^{bdac}
4ms-189-5	0.00^{d}	0.00^{d}	0.00^{d}	25.30^{ebdac}	28.2^{bdac}	28.80^{bdac}
4ms-295-9	43.10^{bac}	47.90^{bac}	58.60^{ba}	10.60^{ebdc}	14.60^{ebdc}	16.80^{ebdac}
W0001265	44.64^{bac}	56.20^{ba}	58.00^{ba}	37.00^{a}	42.00^{a}	43.70^{a}
PI196160	40.80^{bac}	46.50^{bac}	47.10^{bac}	18.90^{ebdacf}	21.00^{ebdac}	21.60^{ebdac}
Mean	30.24	36.99	40.05	20.5	24.02	25.59
P	0.007	0.007	0	0	0	0.001

Means's with different letters in the same column are significantly different at P = 0.05

Root length and fresh weight

In this study the root length and fresh weight measured at 15 days after planting to monitor the changes in two traits as consequence of differences in day conditions used. Analysis of root length showed the line 3ms097-3 as less-sensitive to short day condition. This line showed significant difference in root length to the sensitive control PI196160 in short day condition (Table 5). Also, 3ms097-3 showed less changes compared to the less-sensitive control W0001265 (Table 5). The line 3ms097-3 showed neutral response to day length expressed in fresh weight. Variation in environmentassl factors such as temperature, photoperiod and moisture is possible to cause a negative impact on plant growth and metabolism [35,36]. Studies in Pycnosorus thompsonianus grown under long daylength (LD) produced number of inflorescences per plant two times more than the plants grown under SD (79.8 vs 33.7) [37].

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Table 5: Root length and fresh weight of plants grown in standard and long day conditions.

Designation	Standard Day condition		Short Day condition	
	Root length (cm)	fresh weight (gm)	Root length (cm)	fresh weight (gm)
qiu 1	9.30^{jhi}	2.40^{b}	9.80^{c}	1.98^{gf}
qiu 2	11.20^{de}	1.88^{edf}	9.20^{de}	2.06^{fe}
3ms-089-7	12.50^{c}	2.40^{b}	9.20^{de}	2.17^{dce}
3ms-095-6	11.20^{de}	2.07^{cd}	9.30^{de}	2.12^{de}
3ms-097-3	14.30^{b}	3.07^{a}	12.70^{a}	2.90^{a}
3ms-102-5	12.20^{c}	1.99^{cd}	9.80^{c}	2.28^{c}
3ms-104-6	10.00^{ghf}	1.35^{ji}	9.30^{de}	1.87^{gh}
3ms-113-6	11.00^{de}	1.65^{hgf}	0.0^{k}	0.00^{l}
4ms-146-1	11.60^{dc}	1.90^{ed}	9.30^{de}	1.56^{j}
4ms-147-5	10.30^{gef}	1.5^{hjgi}	0.00^{k}	0.00l
4ms-148-4	10.00^{ghf}	1.45^{hji}	0.00^{k}	0.00l
4ms-156-2a	8.20^{kl}	2.00^{cd}	6.50^{i}	1.76^{i}
4ms-156-5	8.40^{jkl}	1.70^{egf}	9.20^{de}	2.19^{dc}
4ms-161-10	10.00^{ghf}	1.04^{k}	9.60^{dc}	2.07^{dfe}
4ms-161-3	9.10^{jkhi}	1.57^{hgi}	4.50^{j}	2.15^{de}
4ms-168-1	7.60^{l}	1.01^{k}	8.30^{g}	1.16^{k}
4ms-183-9	9.40^{ghi}	1.41h^{ji}	9.00^{fe}	1.85^{ih}
4ms-184-4	8.80^{jki}	1.28^{j}	8.50^{g}	1.11^{k}
4ms-189-5	0.00^{m}	0.00^{l}	9.00^{fe}	1.89^{gh}
4ms-295-9	10.80^{def}	2.18^{cb}	8.60^{fg}	1.91^{gh}
W0001265	18.00^{a}	3.13^{a}	10.60^{b}	2.55^{b}
PI196160	9.30^{jhi}	1.88^{edf}	7.50^{h}	1.97^{gfh}
Mean	10.15	1.77	7.72	1.71
P	<.000	<.000	<.000	<.000

Means's with different letters in the same column are significantly different at P = 0.05.

Cluster analysis

Variability is a main factor that leading to better grasp of knowledge of genetic frameworks and characterization of genomic regions conferring important traits. The successful breeding program creates choice for parent selection by developing high level of variability in the utilized germplasm. In this context we developed mutant lines varied in response to day conditions. In this study 22 mutant genotypes were observed for seven phenotypes as; stem determination, number of leaflets, leaf shape, pubescence presence or absence, pubescence color, pubescence density and pubescence type. The cluster revealed that the 22 genotypes structured into three major groups with six subgroups (Figure 2). The four major groups distributed as; the first group comprises seven genotypes formed two subgroups with five sub-subgroups (Figure 2). The first group includes 3ms104-6 and 4ms-183-9 which detected as plant height neutral and less- sensitive respectively to the two days conditions at early growth stage. The second group also comprises seven genotypes formed two subgroups with four sub-subgroups (Figure 2). The second group includes 3ms-097-3 and 3ms95-6 which detected as neutral in germination and fresh weight respectively. The third group comprises eight genotypes formed two subgroups with four sub-subgroups (Figure 2). The third group includes the 3ms089-7 was detected as neutral short day expressed in fresh weight. Our result showed less number of groups when compared to forty genotypes of soybean grouped into nine clusters when assessed for chlorophyll aspects and seven clusters when précised for yield characteristics [38]. The line ms097-3 detected as neutral response to short day conditions located in group two [39-41].

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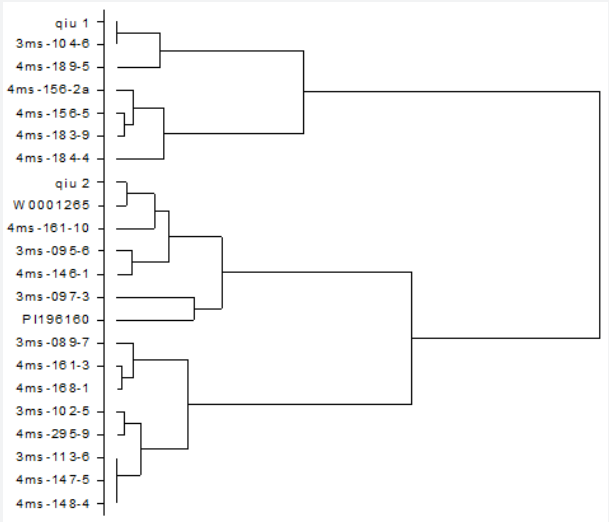

Figure 2: Cluster analysis of the tested soybean mutant lines generated from seven morphological aspects including stem determination, number of leaflets, leaflet shape, pubescence, pubescence density, pubescence color and type. Lines structured in three main groups.

Conclusion

The experiment was designed and carried out to develop new sources of less-sensitive to short day conditions. Through this work the line 3ms097-3 identified as less-sensitive to short day cues expressed in plant germination, plant height, root length and fresh weight respectively. The plants grown in short day length condition showed shorter plant height and root length and also low number of fresh weights. Short day condition is unfavorable for soybean plants to produce long roots, shoot and to develop good plant stand. The detected line could be advanced for field testing before utilization in breeding purposes.

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