Sulfur Fertilization Influence on Growth and Yield Traits of Three Korean Soybean Varieties

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Abstract: Field experiment was carried out at the Research Farm of Korea University, Republic of Korea to study effects of sulfur (S) fertilization on growth traits, yield and yield components of three Korean soybean varieties (Poongsunnamulkong, Saedanbaekkong and Daewonkong). Seeds were sown on black polythene mulched soil bed at the spacing of 15 X 60 cm both at control and sulfur fertilized (gypsum with 16% sulfur content at the rate of 200kg ha^{-1}) plots following a Randomized Complete Block Design of the experiment applying three doses of sulfur together at the time of seed sowing and two doses together at V4 vegetative growth stage (4 nodes on the main stem beginning with the unifoliate node). The results showed that sulfur fertilization significantly increased all studied growth traits of three Soybean varieties except plant height when they all reached physiological maturity stage (120 DAS). It was also evident that sulfur fertilization in the form of gypsum can also increase the number and weight of soybean root nodules and enhance the ability of nitrogen fixation, and so reduce the input of nitrogen fertilizer. Similarly, all the studied yield and yield component related parameters were also significantly influenced by sulfur fertilization except seed number per pod and 100 seed weight. Varieties and sulfur fertilization also interacted significantly in all studied growth traits but the notable sole interaction effect was in plant height. Leaf area of Poongsunnamulkong and Saedanbaekkong variety increased significantly with sulfur fertilization while it was decreased significantly for Daewonkong. Similar trend was observed in their production of number of pods per plant and grain yield per plant indicating leaf area increment by sulfur fertilization played an important role in increasing yield of Poongsunnamulkong and Saedanbaekkong varieties though their performance was reverse in other growth traits. The improvement in growth and yield attributes after sulfur fertilization led to higher biological yield and enhanced seed yield.

Keywords: Soybean; Gypsum; Poongsunnamul kong; Saedanbaek kong; Daewon kong

1. Introduction

Sulfur deficiency has become a limiting factor for crop yield in many areas of the World (Mascagni *et al.,* 2008). Sulfur is becoming deficient in soils due to introduction of high yielding varieties, use of high grade S free fertilizers and reduced emission of S from industrial units (Scherer, 2009).

Sulfur is increasingly being recognized as the fourth major plant nutrient after nitrogen, phosphorus and potassium. The importance of S in agriculture is being increasingly emphasized and its role in crop production is well recognized (Jamal *et al*., 2005; 2006a; 2006b; 2006c; 2009; 2010a; Scherer, 2009). Sulfur is one of the 16 elements essential for plant growth and is a component of amino acids (methionine and cysteine) needed for protein synthesis (Jan *et al.,* 2002). Sulfur is also a vital part of the ferredoxin, an iron-sulfur protein occurring in the chloroplasts. Sulfur plays important role in vegetative growth of plant as it is a component of ferredoxin in chloroplast and is involved in photosynthetic process (Fukuyama, 2004). Ferredoxin has a significant role in nitrogen dioxide and sulfur reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer *et al*., 2008).

Average S removal for producing 1 ton of food grain is estimated to be 3-4 kg by cereals (wheat & rice), 5-8 kg by sorghum and millet, 8 kg by pulses and legumes and 12 kg by oilseeds (Kanwar and Mudahar, 1985).

Among the fertilizer elements sulfur requirement of oilseed crops is quite high as compared to other crops (Das and Das, 1994). To overcome the problems associated with S deficiency, a number of S-containing fertilizers as well as other S containing by-products from industrial processes are available (Jamal *et al*., 2010b). Application of sulfur improved nitrogenase activity, nitrogen fixation, plant dry matter in sulfur deficient soil in the study conducted by Kandpal and Chandel (1993).

Sulfur application has immense potential of increasing the amount of N fixed by legumes, thus improving fertility status of soil (Habtemichial *et al.,* 2007). Sulfur availability has a role in regulating nitrate reductase and ATP-sulphuurylase and increases protein and chlorophyll content in legumes (Jamal *et al.,* 2006b and 2010a; Ahmad *et al.,* 2007).

Soybean is a sulfur loving plant and like other oilseed crops, its sulfur requirement is more than those of many other crops for proper growth and yield. The response of soybean to sulfur application has been reported by Nagar *et al*. (1993), Rao and Ganeshamurthy (1994), Joshi and Billore (1998). It promotes nodulation in legumes and gives bold seeds in oil-seeds. Moreover, application of sulfur enhances uptake of N, P, K and Ca by the crop (Nasreen and Farid, 2006). Many works have been carried out in South Korea (Kwon *et al*., 1974; Won *et al*., 1983; Moon *et al*., 1985;Yoon *et al*., 2003; Cho *et al*., 2006; Shin *et al*., 2007; Ok *et al*., 2008) and Indonesia (Syaiful *et al*., 2014) on the growth and yield of soybean crops, but a very few of them (Lim and Eom, 1984; Jamal *et al*., 2005) studied effect of sulfur fertilization on growth characteristics and yield of soybean varieties and none of them studied influence of solely sulfur fertilization and compared growth characteristics of Korean soybean varieties at different stages of their life cycle though sulfur fertilization has been experimentally proved promising for growth and productivity of soybean in many parts of the World. Since sulfur plays a vital role in numerous metabolic pathways and impacts plant productivity and nutritional quality (Yi *et al*., 2010), the present study aimed to explore effect of sulfur fertilization on growth and yield traits of Korean soybean variety Pungsannamulkong at different stages of their life cycle.

2. Materials and Method

Field experiment was conducted during the summer in conventional agricultural farm soil of Republic of Korea located between 37º34′ N Longitude and 127º13′ E Latitude to 37º35′ N Longitude and 127º14′ E Latitude and at an altitude of about 678 meters above the mean sea level. Mean monthly temperature and total monthly precipitation for the studied growth period of Soybean (June to October, 2012) for Namyangju region of Republic of Korea have been shown in Figure 1. The experiment was laid out in the farm area following a randomized complete block design with two factors. Six treatment combinations, combining two treatments (control and Sulfur fertilization) and three Korean soybean varieties (Pungsannamul kong, Saedanbaek kong and Daewon kong) were randomly assigned in each of the four experimental blocks. Total 24 rectangular experimental plots were finally laid out with gross plot size 0.9 x 2.78 m with net plot size 0.6 x 2.78 m. Black polyethylene mulch was placed on firmly pressed bed and Gypsum with 16% Sulfur content was applied in shallow furrows at the middle of two lines at the rate of 200kg ha^{-1}; three cumulative doses together at the time of seed sowing and two cumulative doses together at V4 vegetative growth stage (4 nodes on the main stem beginning with the unifoliate node) according to Fehr *et al*. (1971).

Planting positions were marked on black polythene mulch according to desired spacing (15 X 60 cm) for soybean and all holes in an experimental plot were sown by seeds of a specific soybean cultivar. Drip watering was provided with drip tape up to almost soil water saturation level immediately after seed sowing. After one week of germination of seeds, thinning of germinated seedlings was carried out just to keep a single and the best seedling in each planting position. Drip irrigation was continued once a week up to six weeks as there was almost no rain during that period. Weeding was done at the base of the plant at regular interval of one week. Plant protection measures were taken against various insects and diseases. High tunnel/ high hoop/hoop house was constructed in the field in order to protect soybean seeds from birds before germination and to protect crops from the pests after germination.

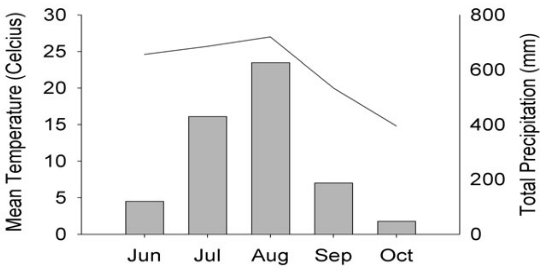

Figure 1. Mean monthly temperature and total monthly precipitation for the studied growth period of Soybean (June to October, 2012) at Namyangju region of Republic of Korea (Source: RDA Agricultural weather information service http://weather.rda.go.kr)

2.1 Growth Traits

For growth traits, biometric observations were recorded on three randomly selected plants from each plot at physiological maturity stage (120 DAS). Plant height was measured in centimeter from the base of the plant to the tip of the tallest leaf for three randomly selected plants and the mean of three plants was expressed as plant height in centimeters. Leaf area per plant was measured by weight apportionment method described by Horchani *et al*. (2008).

The selected plants were finally carefully uprooted and soil from the root was eliminated. The number of nodules was counted per plant basis and their average was taken as the number of nodules per plant. The sampled nodules as well as roots were first air dried and then oven dried at 70ºC for 72 hours to constant weight. Root dry weights were measured first then the dry weight of the nodules was also measured and recorded. Other plant parts (leaf, shoot and root) of the sampled plants were also first air dried and then oven dried at 70ºC for 72 hours to constant weight to get leaf dry weight and shoot dry weight.

2.2 Chlorophyll Content

Total chlorophyll content in leaves was measured at mid flowering stage following the method of Arnon (1949). Fresh leaf samples (0.05g) collected from the field were macerated in laboratory in turgid condition in mortar and pestle with 80% acetone. It was then collected in a test tube and centrifuged at 3000 rpm for 10 minutes. The volume was made to 10 mL with 80% acetone and the absorbance was measured at 663 nm and 645 nm in a UV/VISIBLE Spectrophotometer (ULTROSPEC 6300 PRO; Amersham Biosciences).

2.3 Yield and yield components

Three other plants were randomly selected each time from each plot at reproductive growth stages (Fehr *et al*., 1971) R5, R6, R7 and R8. Pod Length of three pods from each of the selected plants was measured in the field. Seed length and width was measured in laboratory in fresh condition. Plants were cut at the ground level and kept in separate paper bags. Pod number per plant and seed number per pod were counted. Grain yield (dry weight of seed), straw yield (dry weight of straw) were determined (g/plant) and 100 seed weight were measured after oven drying at 70ºC for 72 hours. Total above ground dry weight was calculated also adding straw yield with grain yield. Harvest Index (HI) was calculated according to following formulae and was expressed in percentage.

HI = Grain yield / (Grain yield + Straw yield)

Yield values were first adjusted to a fixed percentage of moisture content (At 12 % moisture content for cereals) according to the following equation (Ali and Talukder, 2008):

$$Y_{adj} = Y_i X (100+M_t) / (100+ M_i)$$

Where:
M_i is the initial moisture content
Y_i is the initial yield (at M_i moisture content)
M_t is the targeted moisture content (12%)
Y_{adj} is the adjusted yield (at M_t % moisture content)

2.4 Data Analysis

Treatment differences were explored by analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) test. The statistical software MSTAT was used for data analysis.

3. Results and Discussion

3.1 Effect on Growth Traits and Total Leaf Chlorophyll

Sulfur fertilization significantly increased all studied growth traits (Leaf area, Leaf dry matter, Number of root nodules, Nodule dry matter, Root dry matter, Shoot dry matter, Shoot to root ratio, Total plant biomass) of three soybean varieties (Figure 2 and 3) except plant height when they all reached physiological maturity stage (120 DAS). This result of sulfur fertilization significantly influencing growth traits is consistent with the finding of Zhao *et al*. (2008) especially for root dry weight, number of root nodules and nodule dry matter though their studied soybean variety was Yudou No. 25 and the form of sulfur fertilizer was elemental sulfur. The above results show that sulfur fertilization in the form of gypsum can also increase the number and weight of soybean root nodules and enhance the ability of nitrogen fixation, and so reduce the input of chemical nitrogen fertilizer. Sulfur is a vital part of the ferredoxin, an iron-sulfur protein occurring in the chloroplasts. Sulfur plays important role in vegetative growth of plant as it is a component of ferredoxin in chloroplast and is involved in photosynthetic process (Fukuyama, 2004). Ferredoxin has a significant role in nitrogen dioxide and sulfphate reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer *et al*., 2008).

The present result for plant height is not consistent with the finding of Nasreen and Farid (2006) where they obtained tallest plants from sulfur application while it was relatively shorter in control plots (no sulfur added) though their studied soybean varieties were Bangladeshi (Sohag and Bangladesh Soybean-4) and the study place was Bangladesh. This inconsistency may be due to GE interaction.

Sulfur fertilization significantly increased total leaf chlorophyll content of leaf samples collected at mid flowering stage irrespective of varieties (Table 1). This result of increment of total leaf chlorophyll content with sulfur fertilization is consistent with the findings of Zhao *et al*. (2008).

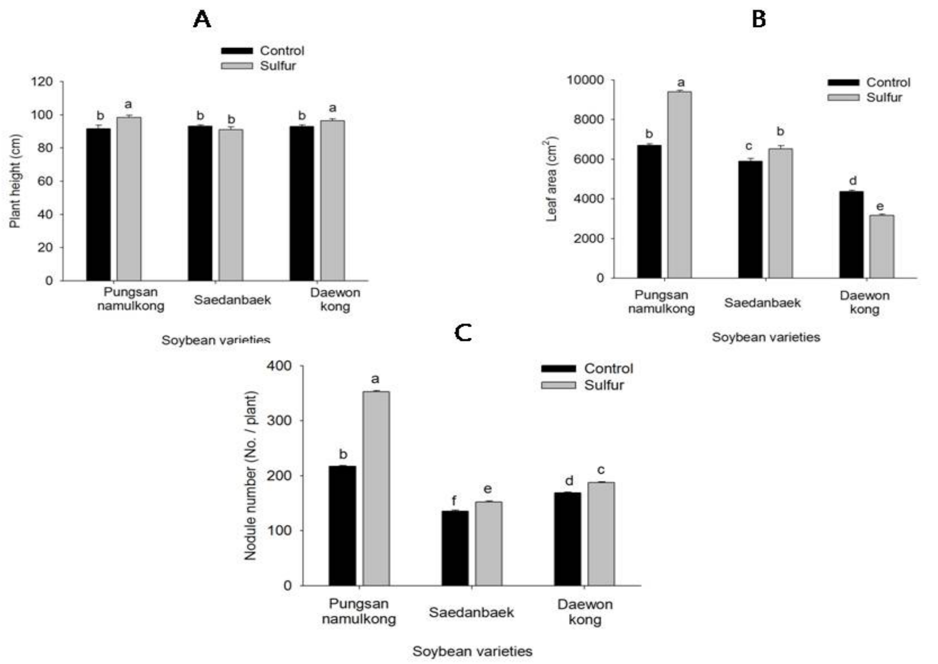

Figure 2. Difference in height growth, leaf Area and nodule number between control and sulfur treated Soybean plants of three Korean varieties at their physiological maturity stage (120 DAS)

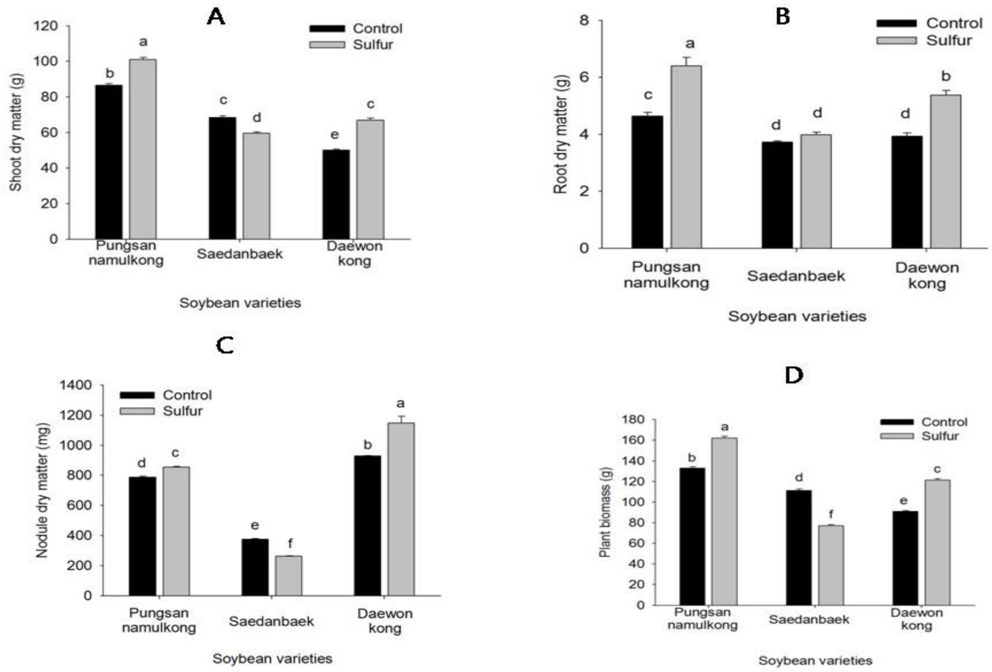

Figure 3. Difference in shoot dry matter, root dry matter, nodule dry matter and also in plant biomass between control and sulfur treated Soybean plants of three Korean varieties at their physiological maturity stage (120 DAS)

Table 1. Chlorophyll content of leaf at mid flowering stage

Description of factor											
Treatment		**Variety**									**Interaction**
Control	Sulfur	Pungsunnamulkong			Saedanbaek kong			Deawonkong			Treatment x Variety
		Control	Sulfur	Mean	Control	Sulfur	Mean	Control	Sulfur	Mean	
5.05b	6.27a	4.59b	7.46a	6.03b	4.94a	4.68b	4.81c	5.62b	6.67a	6.14a	*

3.2 Effect on Yield and Yield Components

For all three Soybean varieties, the studied yield and yield component related parameters were also significantly influenced by sulfur fertilization (Figure 4 and 5) except seed number per pod and 100 seed weight. This result of sulfur fertilization significantly influencing grain and dry matter yield is consistent with the findings of Lim and Eom (1984) but the studied Korean soybean variety was *Gwanggyo* and the applied form of sulfur was not gypsum.

This result of sulfur fertilization significantly influencing yield and yield attributes is also consistent with the finding of Nasreen and Farid (2006) though their studied soybean varieties were Bangladeshi (Sohag and Bangladesh Soybean-4) and the study place was Bangladesh. This result of higher yield is also consistent with the findings of Zhao *et al*. (2008) though their studied soybean variety and the form of sulfur fertilizer were different as they used the variety Yudou No. 25 and the sulfur was elemental sulfur.

3.3 Interaction Effect of Varieties and Sulfur

Varieties and sulfur fertilization interacted significantly in all studied growth traits when soybean plants reached their physiological maturity stage (120 DAS) but the notable sole interaction effect was in plant height as plant height of Poongsunnamulkong and Daewonkong varieties significantly increased absolutely for interaction effect while it was significantly decreased for Saedanbaekkong. This result of significant interaction effect of varieties and sulfur fertilization on plant height is consistent with the findings of Hasan and Kamal (1998) though they did not apply sulfur fertilizers and the varieties were wheat varieties. Interaction effect of variety and different type manure and fertilizer also significantly affected plant height of *Brassica oleracea* L. (Cabbage) in a study by Hasan and Solaiman (2012) in Bangladesh.

Similarly, all the yield and yield component related traits were also significantly influenced by interaction effect but the notable sole interaction effect were for seed number per pod and 100 seed weight. Due to significant interaction effect of varieties and sulfur fertilization, seed number per pod for Pungsunnamulkong variety has increased significantly, while 100 seed weight has also significantly increased for Saedanbaekkong. Seed yield is a complex characteristic that includes various components and at last results in high yield. Diepenbrock (2000) reported that seed weight of a pod is the product of the number of seeds per pod and the mean weight of individual seeds. Seed weight and pods per plant are usually negatively correlated and further a negative relationship had often been found between seed weight and number of seeds per pod (Leaon and Becker, 1995).

Leaf area of Poongsunnamulkong and Saedanbaekkong varieties increased significantly with significant effect of sulfur fertilization as well as for significant interaction effect of varieties and sulfur fertilization while it was decreased significantly for Daewonkong. Similar trend was also observed in their production of number of pods per plant and grain yield per plant indicating that leaf area increment by sulfur fertilization and interaction effect played an important role in increasing yield of Poongsunnamulkong and Saedanbaekkong varieties though their performance was reverse in other growth traits. Yield formation in grain crops involves protein, oil, and carbohydrate synthesis by actively growing seeds, dependent on raw materials supplied by the mother plant in the form of C and N assimilates. In soybean, photosynthesis during the seed filling period (SFP) appears to supply most of the C needed for seed development (Shibles *et al*., 1987; Wardlaw, 1990), and sucrose is the most abundant form of C in the phloem and the predominant C source for developing seeds (Layzell and LaRue, 1982; Rainbird *et al*., 1984).

The capacity of the plant to produce an economic yield depends not only on the size of the photosynthetic system, its efficiency and the length of time for which it is active but also on translocation of dry matter into seed yield. Besides, an increased supply of photosynthates to pods would also provide an opportunity for seeds to grow to their full potential, with an obvious increase in 100-seed weight observed for both Poongsunnamulkong and Saedanbaekkong in our study though the increment was significant only for Saedanbaekkong.

The improvement in growth and yield attributes after sulfur fertilization led to higher biological yield and enhanced seed yield. After flowering, no further leaf expansion is possible and no improvement in yield potential of the crop can be achieved, so the application of S before flowering is beneficial in improving both photosynthetic efficiency and yield potential of the crop. Inadequate supply of nutrients (S and N) at flowering time decreases not only the photosynthetic area and its duration but also the photosynthetic rate (Ahmad *et al*., 1999). Reduced availability of photosynthates can restrict the development of floral buds as well as the development of newly formed pods and seeds (Ahmad *et al*., 1999).

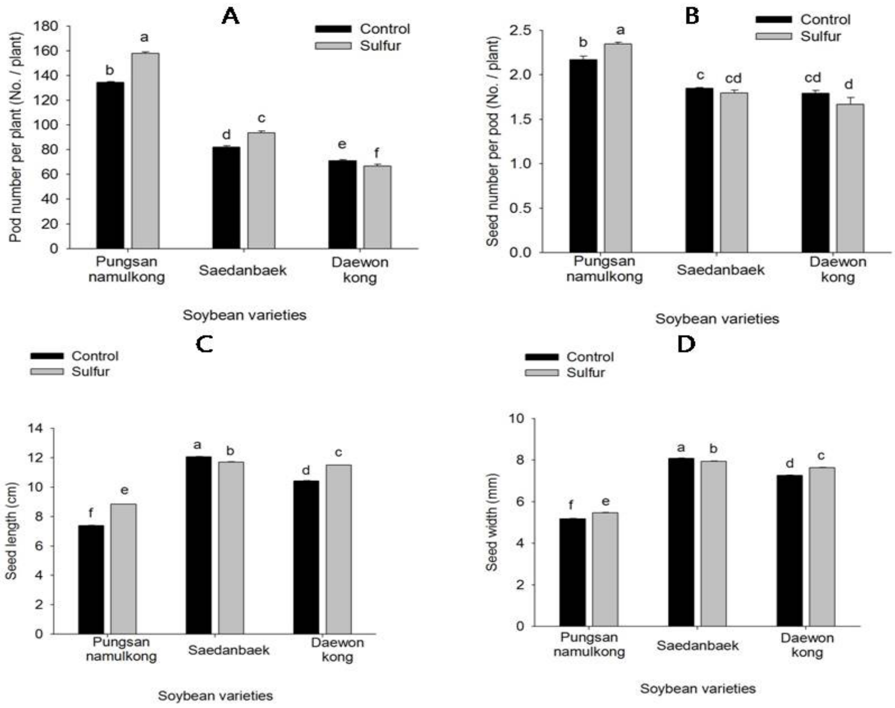

Figure 4. Difference in pod number, seed number, seed length and seed width between control and sulfur treated Soybean plants of three Korean varieties at their harvest maturity

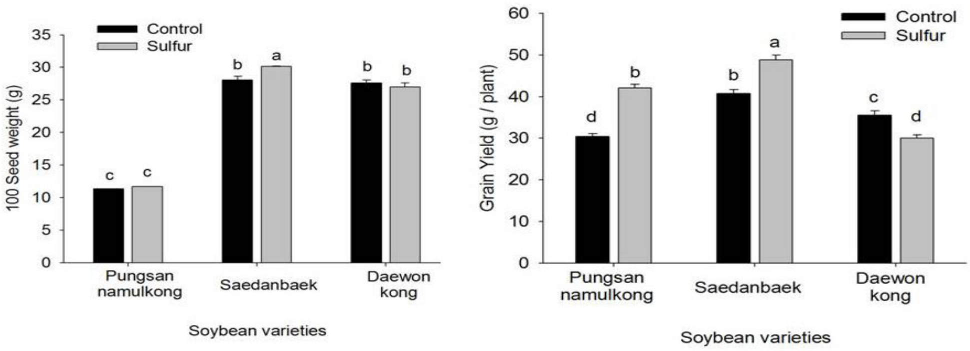

Figure 5. Difference in Grain yield between control and sulfur treated Soybean plants of three Korean varieties at harvest maturity

4. Conclusion

Sulfur fertilization significantly increased nodule number per plant compared to control for all three varieties. Application of sulfur in the form of gypsum significantly increased value of most of the studied growth traits for Poongsunnamulkong and Daewonkong varieties while those decreased significantly for Saedanbaekkong except for root dry weight. Similar trend was also observed for total leaf cholorophyll content at mid flowering stage.

Leaf area of Poongsunnamulkong and Saedanbaekkong varieties have increased significantly with sulfur fertilization while it was decreased significantly for Daewonkong. Similar trend was observed in their production of number of pods per plant and grain yield per plant indicating that leaf area increment by sulfur fertilization played an important role in increasing yield of Poongsunnamulkong and Saedanbaekkong varieties though their performance was reverse in other growth traits. The improvement in growth and yield attributes after sulfur fertilization led to higher biological yield and enhanced seed yield.

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