

Influence of Herbicides Application as Desiccant on Protein and Sugar Content of Soybean Seed

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Abstract
Soybean, *Glycine max* is an annual crop belonging to the family *Fabaceae,* which has 40% protein, 20% oil and 30% carbohydrate and plays a very significant role in world agriculture. Some farmers hasten the maturity and desiccation of soybean crop by applying herbicides without giving attention to the effects on seed. This study was conducted to investigate the effect of herbicides as desiccant on protein and sugar content of soybean seed. For this, three cultivars of soybean seed SB 19, SB 25 and SB 3 were planted and applied with three types of herbicides Glyphosate, Paraquat, 2,4-D. The herbicides were sprayed at three stages of soybean seed development R6, R7, and R8. The herbicides effect was highly significant ($P \leq 0.001$) on the protein content in two locations and were highly significantly different ($P \leq 0.001$) on the sugar content in all locations. All the herbicides showed reduction in sugar content in all locations and were also lower in protein content as compared to the control. It is concluded that using herbicides as desiccant have negative effect on the protein and sugar content of soybean seed.
Keywords: Herbicide, Stages of application, Protein, Sugar.
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1. Introduction

Soybean, *Glycine max* is an annual herbaceous leguminous crop belonging to the family *Fabaceae,* which has erect bushy leafy plant structure. It is a strictly self-pollinating legume with 40% protein, 20% oil and 30% carbohydrate and plays a very significant role in world agriculture (Tefera, 2010). Due to its high protein level, about 98% of the soybean meal is used in livestock and aquaculture feeds. A smaller percentage is processed to make soy flour and protein for human consumption. An even smaller percentage is used as a fresh vegetable known as “mao dou” in China, “edamame” in Japan, and green vegetable soybean or edamame in the USA and other countries (Shanmugasundaram and Yan 2010). Seed development, maturity, and harvest management are critical considerations for maintaining high seed quality (Bewley *et al*., 2013). Kumar *et al*. (2002) reported that seed quality largely depends on the stage of maturity of crops. Therefore, harvesting of seeds at the optimum stage of maturity is important as harvesting either at early or late stage results in poor quality seeds.

Herbicides are metabolic inhibitors and their mode of action can be classified in to different groups; Photosynthetic inhibitor, Cell growth disrupters (mitotic inhibitors), Growth regulators, Lipid biosynthesis inhibitor, Carotenoid biosynthesis inhibitor and Branch chain amino acid inhibitor (Hess, 1999). Herbicide may be classified according to selectivity; as grass control, broad leaf control, pre-emergence or post emergence and persistence (Peterson *et al.,* 2001). Also, their ‘modes of action’ ultimately prevent plants from making essential carbohydrates, proteins or lipids (oils and fats); or desiccate leaves and stems, Center (2018).

Franz *et al*. 1997 reported that, glyphosate inhibits 5- enolpyruvyl-shikimic-acid-3-phosphate synthesis and thus prevents amino acid biosynthetic pathways; and inhibition of amino acid biosynthesis by glyphosate may block the accumulation of proteins in seed (Mirzan *et al*., 2003). This will in turn lower reserve protein content when applied prior to physiological maturity. Delgado *et al*. (2015) reported that, the application of glufosinate ammonium as desiccant promoted the reduction of the initial soluble sugar content in seeds of two cultivars of soybean in relation to their controls.

Chen *et al*. (2003) reported that the herbicide alachlor absorbed by emerging plant shoots, inhibits biosynthesis of fatty acid and lipid; as well as synthesis of gibberellins and protein. In studying the effect of herbicides on green gram plants, the protein content in grains was found to be severely affected by the higher dose rates of atrazine and Isoproturon, suggesting that the enzymes and other functional proteins are one of the target sites of herbicide toxicity, which subsequently leads to alteration in the protein metabolism of grains (Khan *et al*., 2006). In another research on wheat Kumar (2012) indicated that both herbicides tested were able to reduce the carbohydrate and protein content gradually from lower to higher concentration. The seed protein was also reported to differed significantly due to various treatments of herbicides sprayed at different crop growth of soybean (Neelu, 2015).

However, there has been little information about the herbicides effect on sugar content of soybean seed after desiccating the plant with herbicides. This study therefore determines the effect of herbicides on protein and sugar content of soybean seed.

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2. Materials and Methods

Three varieties of soybean seed SB 19, SB 25 and SB 3 obtained from CIAT–TSBR Maseno Kenya were planted at three locations in the 2017 raining season. The locations were Busia ATC; tropical climate, temperature 22ºc, annual rainfall 1691mm, warmest March, August, coldest. The soil type is loamy, strongly acidic (pH 4.97). Mabanga ATC; tropical climate, temperature 21.5 oC, annual rainfall 1618mm, warmest March and coldest July. The soil type is sandy loam, strongly acidic (pH 5.22). and University of Eldoret; climate is warm and temperate, Temperature 16.8ºc, and annual rainfall 1055mm. The soil type is sandy - clay loam, strongly acidic (pH 5.22).

2.1 Field Experiment

The experiment was laid as split – split plot arrangement in RCBD replicated three times in each location. The herbicides were Glyphosate (GLY), Paraquat (PRQ), 2,4-D and the control (C) in which no herbicide was applied. The herbicides were sprayed at three stages of soybean seed development R6 is when pods contain green seeds that fill the pod to capacity at one of the four uppermost nodes on the main stem, R7 is beginning of maturity one pod on the main stem has reached its mature color (tan, brown, or black), and R8 is defined as full maturity. Ninety-five percent of the pods have reached their mature color, as described by (Wright and Andrew, 2013). All the herbicides were applied at the rate of 2L ha^{-1}. The seed harvested were air dried and threshed. The samples were grounded and subjected to soluble sugar and protein analysis.

2.2 Soluble Protein Determination

The soluble protein was determined using modified Lowry protein measurement according to the method described by Hartree (1972). The assay involves diluting the extract to 1ml, with H_2O and adding 0.9 ml of solution A before incubation for 10min at 50 ºC. The samples were cooled down to room temperature, added 1 mL of solution B in 0.1 M (NaOH) and left for 10 min. Finally, 3 mL of solution C was added before incubation for 10 min at 50 ◦C. A standard curve was made of bovine serum and absorbance was read and recorded.

2.3 Soluble Sugar Determination

The soluble sugar was determined using Phenol sulfuric acid method according to Bhilai (2015), with some modification. A 0.2,0.4,0.6,0.8 and1ml of working standard (with 10mg/100ml conc.) of glucose was taken in boiling tubes and the final volumes of each tube was made 1ml by adding distilled water. 1ml of 5% Phenol and 5ml of 96% Sulphuric acid was added one by one in each tube and shook well so that the Phenol and Sulphuric acid get mixed thoroughly with working standard. After 10 minutes all the tubes were placed in water bath at 25-30°C for 15 minutes. Blank was set with 1ml of distilled water and optical density of each tube was taken at 490nm with the help of spectrophotometer and the reading was recorded.

2.4 Data analysis

The Analysis of variance was performed for the data collected and further analysis was done using contrast comparison. All analyses were done using the Genstat computer package programme, Genstat 14th edition 2013.

3. Results

3.1 Protein content as affected by herbicides

The cultivars and herbicides performed differently on protein content with respect to locations. The protein content of the cultivars ranges from 30.9 to 41.0 % with SB19 higher in protein. The herbicides treatments were not significantly different in Busia and Mabanga. However, in UoE, they differed significantly ($P \leq 0.05$) in that, they performed poorly compared to control. The protein content between the herbicides treatments ranges from 25.6 to 41.5 % and paraquat treated plots produced the lowest protein percentage in all the locations (Fig. I). lowest protein content was obtained in UoE and highest protein content in Busia.

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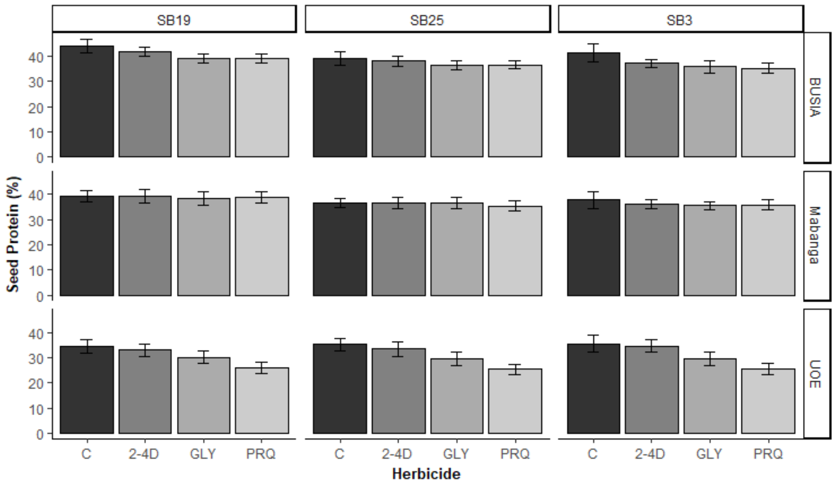

Figure 1. Effect of herbicides on protein content of soybean seed produced at UoE, Mabanga and Busia. Error bars compare the herbicides only.

3.2 Sugar content as affected by herbicides

There was no significant difference among the cultivars on sugar content at UoE but differed significantly ($P \leq 0.05$) at Mabanga and Busia. The sugar content of the cultivars ranges from 17.1 to 24.4 % with SB19 higher in sugar. There were significant differences ($P \leq 0.05$) on sugar content between the herbicides treatments. All the herbicides performed poorly compared to control plots. The sugar content among the herbicides ranges from 16.4 to 25.8% with higher sugar percentage in the control and lower in paraquat treatment (Fig II). The seeds from Mabanga had more sugar content while that of Busia and UoE were statistically similar.

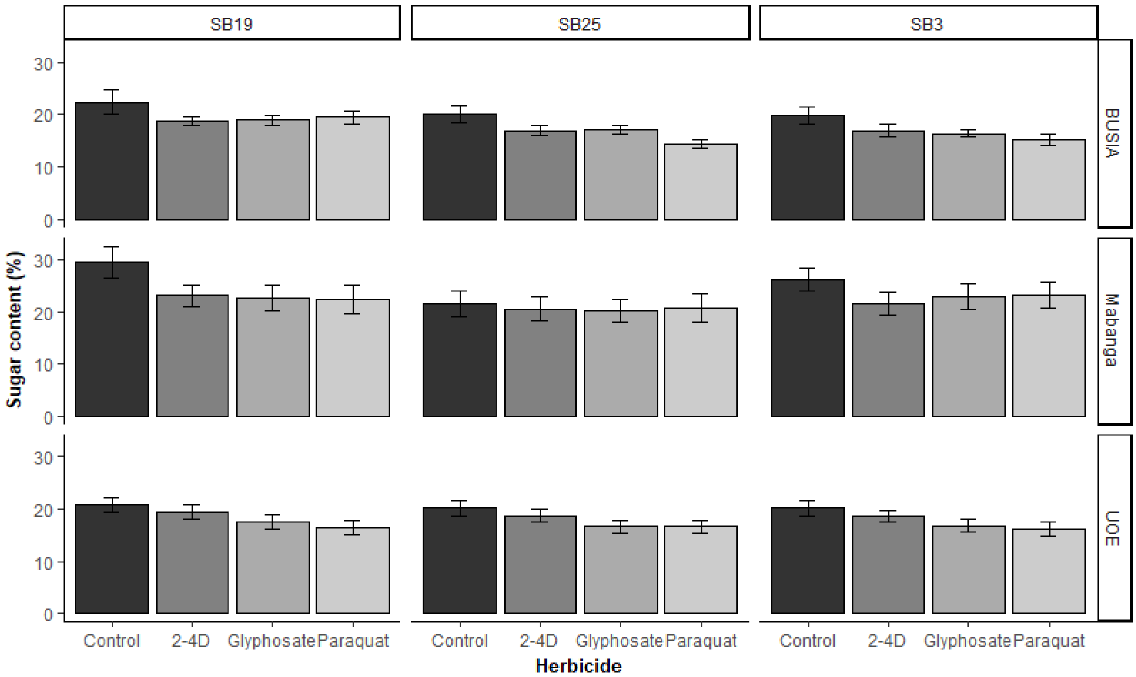

Figure 2.Effect of herbicides on sugar content of soybean seed produced at UoE, Mabanga and Busia. Error bars compare the herbicides only.

3.2 Protein and Sugar content as affected by application stages

The protein content was highly significantly different ($P \leq 0.001$) due to application stages in all locations. It ranged from 23.6% to 43.9% in which, the stage R8 had higher protein content followed by R7 and R6 had lower protein. As in the herbicides treatments, the seeds from Busia had higher protein content though not significantly different from that of Mabanga, UoE had the lower protein content (Table 1). The sugar content was also highly significant ($P \leq 0.001$) in all locations. The stage R8 also had higher sugar content compared to R7 and R6. It ranged from 14.69% to 29.22% with higher sugar content at Mabanga and lower sugar content at UoE (Table 1).

Table 1. Protein and sugar content as affected by application stage regardless of the herbicides

Application stages	UoE		Mabanga		Busia	
	Protein	Sugar	Protein	Sugar	Protein	Sugar
R6	23.6[c]	14.7[c]	31.7[c]	16.6[c]	33.2[c]	15.2[c]
R7	32.7[b]	18.2[b]	36.9[b]	22.8[b]	38.9[b]	18.6[b]
R8	37.1[a]	21.9[a]	43.0[a]	29.2[a]	43.9[a]	20.2[a]
S.E.D	**1.1**	**0.9**	**1.9**	**3.0**	**2.6**	**1.4**

Means followed by the same letter(s) are not statistically different at 5 % level of probability

3.3 Interaction between herbicides and application stages on protein content

Herbicides and stages interaction on protein content was significant ($P \leq 0.05$) only at Busia. Control had higher protein content of 49 % in R8, significantly different compared to 2-4D, glyphosate and paraquat treatments. The R8 was significantly higher in protein content percentage than R7 and R6 across all the herbicides (Figure 3).

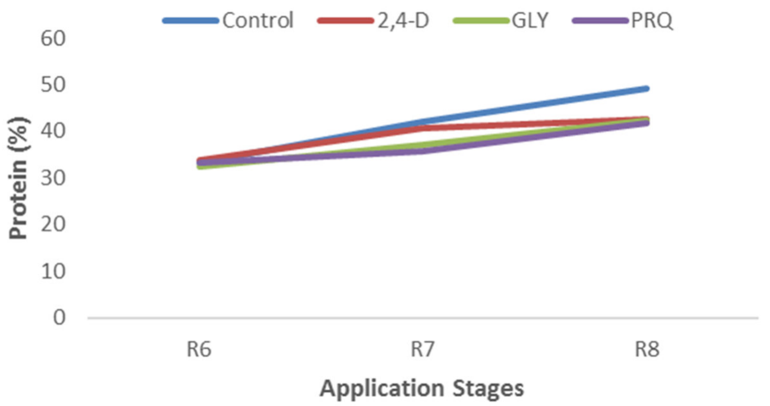

Figure 3.Herbicides and application Stages interaction on Protein content % at Busia

3.4 Interaction between herbicides and application stages on sugar content

Herbicides and stages interaction on sugar was significant ($P \leq 0.05$) only at UoE. Control with 24 % sugar in R8 stage was significantly higher compared to 2,4-D, glyphosate, and paraquat. Glyphosate and paraquat were similar in protein content at R7. Paraquat treatment which had 13 % in R6 stage had lower sugar content. The R8 was significantly higher in sugar content across all the herbicides (Figure 4).

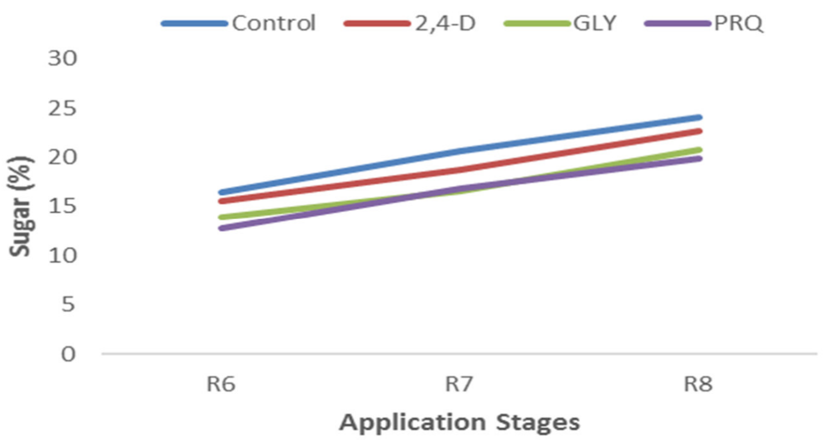

Figure 4.Herbicides and application Stages interaction on sugar content % at UoE

4. Discussion

This study revealed that, seed storage protein pattern is considered as the genotypic fingerprint since the cultivars did not differ on protein content in all the locations. This was confirmed by their initial protein content since they did not differ significantly as SB 19 had 42%, SB25 had 40% and SB3 also had 40% protein genetically. Tariq Shah *et al*. (2017) reported that, no considerable difference was observed between the cultivars of soybean concerning percent protein under different time of sowing of diverse cultivars.

Herbicides application significantly reduced the soluble protein content of soybean seed with higher protein content in control compared to the herbicides treatments. Paraquat treatment recorded lowest in protein content in all the three sites/cultivars. The reduction in protein associated with paraquat could be due to its mode of action which rapidly kills all the tissues that get contact after application whether the seed matured or not. However, 2,4-D and glyphosate are systemic in that, they must translocate to the site of action before phytotoxicity can occur. Herbicides application was also reported to reduce grain protein in green gram seed (Khan *et al*., 2006). They further added that, maximum reduction in grain protein (GP) was observed at 400 µg.a.i.kg^{-1} of isoproturon compared to the control. However, the herbicides type and the rate they used were not the same as lower rate was used in this study hence paraquat at the rate of 200 g ha^{-1} a.i, 2,4-D 600 g ha^{-1} a.i and glyphosate at 480 g ha^{-1} a.i were used in this study.

In another finding by Kumar *et al*. (2012) on wheat it was reported that, the herbicides (2, 4-D and isoproturon) alone or in combination of the two, have effect in terms of total soluble protein. Peer *et.al*. (2013) when using herbicides to control weed in soybean found that pre – planting incorporation of flochlorin 1 kg ha -1 and 1.5 kg ha -1 and pre – emergence of pedimethalin 1 kg ha -1 and 1.5 kg ha -1 reduced protein content as compared to the hand weeding. However, contrary to this finding, Delgado *et al.* (2015) observed that the initial phytate and soluble protein content did not change with the application of glufosinate ammonium, when compared with their controls.

The high temperature, rainfall and relatively long humidity in Busia and Mabanga especially in Busia contributed to the less effect of herbicides and resulted to increase in protein content as compared to UoE. Previous research (Bellaloui *et al*. 2009; Piper and Boote,1999) showed that temperature affects protein and oil concentrations in soybean seed.

The difference on sugar content among the cultivars was also associated with their genetic makeup even though, there was no information on their sugar content. The sugar content was also found to be influenced by herbicides application in all the locations. All the seeds from herbicide treated crops had lower sugar content compared to the control. However, paraquat treatments had the lowest sugar content. The low sugar content in paraquat treatment could also be due to its mode of action which quickly forms a highly reactive compound in the plant that rapture cell membrane causing the fluid to leak out leading to necrosis of the tissues as it is a foliar contact. This inhibits the production of soluble sugar into the seed as there will be no energy transformation into the seeds (Saldivar *et al*. 2011). Kumar (2012) also reported that herbicides (2, 4-D) and Isoproturon (IPU) were able to reduce the carbohydrate content gradually from lower to higher concentration of herbicides on wheat seed. It was also reported by (Vital *et al*., 2017) that glyphosate caused damage to the photosynthetic apparatus and a reduction in the carbohydrate concentration and chloroplastid pigments, with casual damage to cell membranes on sunflower. They emphasized that, this effect was more intense at increased doses.

The herbicides and stage interaction effect on protein and sugar content in Busia and UoE resulted due to the effect of control to paraquat and that of R8 to R6.

5. Conclusions

This study showed that, protein and sugar were influenced by environment, herbicides and stages of application. Highest protein content was recorded in Busia, but Mabanga had highest sugar content. However, UoE was lower in both protein and sugar. Among the herbicides, lowest content of protein and sugar were recorded in paraquat treatments up to 9.5% protein and 4% sugar compared to control. The protein and sugar content were higher in R8 stage and lower in R6 stage.

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