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ANALYTICAL COMPARISON OF AMYLASE ACTIVITIES AND REDUCING SUGARS IN VARIETIES OF SOYBEANS AND COWPEA: IMPLICATION ON INFESTATION BY STORAGE INSECT PESTS

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ABSTRACT

The amount of amylase activities and reducing sugars in seven varieties of soybean (four improved: TGx 1485- 1D, TGx 1440-1E, TGx 1448-2E and TGx 1740-2F) and three local: Landrace 1, Landrace 2 and Landrace 3 varieties and six cowpea (three moderately resistant; (1T 89KD-288, IT 90K-277-2 and IT 95K-207-15) plus three susceptible: (Danborno, Danmisira and Danila) varieties were compared in the laboratory. Amylase activities began earlier (2 hours) in soybean and 4-5 hours later in cowpea hence soybean would respond faster to distortion (e.g. insect infestations) than cowpea. Moreso mean values of amylase activities for cowpea were scattered and not well defined. Reducing sugars in cowpea could be distorted earlier (Day 1) while that of soybean took up to Day 3 indicating that soybean is more stable than cowpea. These two factors may have contributed to making soybean more resistant to insect attack compared to cowpea.

Keywords: Soybean, cowpea, reducing sugars, amylase, storage pests

INTRODUCTION

Soybean, *Glycine max* (L.) Merrill is an important leguminous crop grown in the tropics (Duke, 1990) and is one of the oldest cultivated crops in the world (Weiss, 1983). The crop has versatile usage as food (local and intercontinental dishes); raw materials in industries, livestock feed etc. This is traceable to the high level of protein content of the crop. Branford and Ferries (2000) reported that soybean is presently the most important agricultural commodity on the world market. Present Address: Nigerian Stored Products Research Institute, P. M. B. 1489, Ilorin. Kwara State. Nigeria. Although, there were conflicting information on the level of insect infestation of the crop especially under storage, Okunade (2008) reported that soybean is resistant to Callosobruchus maculatus and *Tribolium castaneum* under storage. This is traceable to physical and chemical factors. Fairely and Kilgour (1963) reported that amylase are polysaccharides which catalyses the hydrolysis of starch or of glycogen into reducing fermentable sugars (maltose) and reducing none fermentable or slowly fermentable dextrinsaband.

Since insects possess digestive amylase that could hydrolyze dietary starch and glycogen into reducing sugar, investigation into the activities of these two important biochemical concepts is important. Hence, this paper discusses comparison of two chemical factors of resistance of soybean: amylase activities and reducing sugars in soybean and cowpea as they affect insect infestation of the crop. Materials and Methods A total of seven varieties of soybean and six varieties of cowpea were used for this investigation.

The soybeans varieties were made up of four improved (TGx 1485-1D, TGx 1440-1E, TGx 1448-2E and TGx 1740-2F) – obtained from International Institute of Tropical Agriculture Ibadan and three local (Farmers) varieties designated as Landrace 1, Landrace 2 and Landrace 3 obtained respectively from local farmers in Kano State, Benue State and Oyo/Kwara States of Nigeria Analysis of the amylase activities and reducing sugars content of the whole grains of these samples was conducted in the Mary Hallway Laboratory, Biochemistry Department, Ahmadu Bello University Zaria as follows: (a) Investigation of amylase activities present in Callosobruchus maculatus About 10 g each of the ground varieties of soybean and cowpea were separately extracted with 250 ml of 40-60% petroleum ether in a separating funnel for three hours.

Thereafter, different volumes of the supernatant solution (95, 110, 120, 130, 140, 145, 155 and 160 ml) were placed in separate flasks and the proteins extracted from soybean and cowpea were washed with water in other to remove the cloudy xanthropropyl present in it which could preclude amylase activities from taking place. Some life adults of the cowpea bruchid seed beetle, *Callosobruchus maculatus* were collected and crushed in buffer phosphate solution with p H7.2 and centrifuged at 280 rpm for 10 minutes to obtain supernatant solution. Each of the protein extracted from soybean and cowpea were placed in test tubes and 1 ml each for the supernatant solution from the insect extract added plus 1 ml each of Bovine Serum Albumin (BSA) to serve as catalyst. Each was replicated three times.

Using the ultra violet spectrophotometer red filter, activities of each sample was taken on hourly basis for five hours at 650 nm. The results obtained were analyzed using SAS (1998 Edition) while means were separated using the SNK at P=0.05. (b) Determination of quantities of reducing sugars (RS) in varieties of soybean and cowpea. One gram each of the separately ground samples of the varieties of soybean and cowpea were dissolved in 100 ml distilled water to make 1.0% solution. Dinitrosalicylic acid (DNS) was prepared by mixing 7.0 g DNS reagent + 0.5 g Rochelles salt + 4.0 g Sodium hydroxide. These were dissolved in 500 ml distilled water and 5 ml of 0.2 M hydrochloric acid. This was used to extract the reducing sugar from the sample. About 2.0 ml of 10% solution prepared earlier were placed in separate tubes each containing 1.0 ml of water and 2.0 ml of DNS. The first test tube was blank while the test tubes for soybean and cowpea were each replicated three times.

The amount of reducing sugar in soybean and cowpea was determined daily for days 1-2 with the aid of ultra violet spectrophotometer at 540 nanometer absorbance, as described by Hallaway (1976). Results obtained were analyzed using SAS (1998 Edition) and mean were separated using SNK at P= 0.05.

RESULTS

The results of amylase activities of soybean and cowpea were presented on Tables 1 and 2.

The result shows that there were significant differences

between the soybean varieties and that of moderately resistant cowpea while susceptible cowpea compared favourably well with the resistant soybeans. When analyzed on hourly basis, there were no significant differences (P=0.05) between hours 1-3 but hours 4 and 5 variously differed from the others (Table 2), indicating different timing in the response of the crop to infestation by insect.

Table 1: Investigation into the effect of the amylase activities of soybean and cowpea varieties

Variety	Mean Amylase activities (540 nm)
A. Soybean	
TGx 1485-ID	0.28bc
TGx 1440-IE	0.27c
TGx 1448-2E	0.29bc
TGx 1740-2F	0.29bc
Landrace 1	0.29bc
Landrace 2	0.36bc
Landrace 3	0.33bc
B. Cowpea (Moderately resistant)	
IT 89KD-288	0.52a
IT 90K-277-2	0.53a
IT 95K-207-15	0.53a
C. Cowpea (Susceptible)	
Danborno	0.36bc
Danmisira	0.35bc
Danila	0.37b
SE ± = 0106	
CV(%) = 23.01	

NOTE: Mean followed by the same letters in the column are not significantly different from one another at (P=0.05).

Table 2: Combined relative quantities of amylase activities in soybean and cowpea varieties based on hours of activities.

Amylase Activities of soybean and cowpea	Hours				
	1	2	3	4	5
	0.425a	0.418a	0.428 a	0.313 b	0.246 c

SE (±) = 0.0632 CV (%) = 23.01

NB**:** Means followed by the same letters in the same column are not significantly different (P= 0.05) from one another.

This indicates that the two crops (soybean and cowpea) had different levels at which insect infestation and damage could take place. From Figure 1, amylase activities began at two hours in soybean and five hours in the two groups of cowpea varieties, indicating that soybean responded faster to distortion than cowpea. (Amylase activities begin where activities are static, that is where the readings are fairly stable.

From Table 3, shows the results for reducing sugars. The mean for soybean differed significantly (P=0.05) from that of moderately resistant cowpea. Again, there were significant differences (P=0.05) in the concentration of reducing sugars of the two groups of cowpea (moderately resistant and susceptible) while the mean values for soybean were not significantly different (P=0.05) from that of the susceptible cowpea.

Table 4 shows the combined effect of daily investigation of reducing sugars in varieties of soybean and cowpea. The result shows that there were significant differences (P=0.05) in the level of activities in the tested crop varieties which increased with days in the tested crop varieties. This is an indication that reducing sugars could only be attacked beyond a particular period.

From Figure 2, the effect of reducing sugar began after Day 3 in soybean (resistant), after Day 1 in Danborno cowpea and after Day 3 in Danmisira cowpea (susceptible). This is a likely indication that reducing sugars could only be attacked by insect pests beyond a particular period.

Table 3: Quantities of reducing sugar in soybean and cowpea varieties

Variety	Mean reducing sugar
A. Soybean	
TGx 1485-ID	0.21a
TGx 1440-IE	0.23a
TGx 1448-2E	0.23a
TGx 1740-2F	0.22a
Landrace 1	0.23a
Landrace 2	0.26a
Landrace 3	0.26a
B. Cowpea (Moderately resistant)	
IT 89KD-288	0.06b
IT 90K-277-2	0.06b
IT 95K-207-15	0.07b
C. Cowpea (Susceptible)	
Danborno	0.27a
Danmisira	0.30a
Danila	0.26a
SE ± = 00.14	
CV(%) = 43.52	

NOTE: Means followed by the same letters in the column are not significantly different (P=0.05).

Table 4: Combined effect of daily investigation of reducing sugars in varieties of soybean and cowpea.

Reducing sugars of soybean and cowpea	Days		
	1	2	3
	0.1451c	0.2054b	0.2610a

SE (±) = 0.067 CV (%) = 43.52

NB**:** Means followed by the same letters in the same column are not significantly different (P = 0.05) from one another.

DISCUSSION

That there were no significant differences between the amylase activities on soybean and cowpea but differing on time (hour) basis implied that small as the amount of amylase activities could be, there are differences in terms of action and activities.

Amylase are polyscaharides which catalyzes the hydrolysis of starch or of glycogen into reducing fermentable sugars (maltose) and reducing none fermentable or slowly fermentable dextrinsaband (Faively and Kilgour, 1963). Amylase activities begin where the readings are to a reasonably extent stationary or fairly stable as that is the best time to start checking the activities in the sample.

In the case of soybean, amylase activities began at about the first three hours while that of cowpea was not well defined and this indicates that amylase in soybean is reasonably stable but scattered/unstable in cowpea. When this is related to insect infestation/resistance, it implies that

if all other factors remains the same, when insect attack both soybean and cowpea, soybean began a counter attack faster (2 hours) and better than cowpea (5 hours) by which time damage may have possibly been done by the attacking insect.

The time and efforts taken by insect to successfully infest cowpea and cause damage will be far higher than that required for soybean. In the absence of "resources" of such magnitude, damage will not be recorded. This is one of the reasons why soybean is resistant to insect attack under storage compared to cowpea.

Results obtained for reducing sugars were similar to those of amylase activities both in terms of quantity and time of activities. Reducing sugars, according to Gove (1993) are sugars that are capable of reducing a mild oxidizing agent (as Fehling solution), thereby, limiting it oxidizing activities e.g. glucose, maltose and lactose.

Reducing sugar, according to Elegbede (1990) are so called because they are not stable and could be destroyed by means of heat (boiling), combustion etc. Consequently, oxidation/reduction reaction is very common with them. From the results, reducing sugar was highest and more stable on Day 3 in soybean and not well defined (scattered) in cowpea.

It is recommended that in future research on this subject with these samples, it could be better to conduct the investigation for only three days in other to obtain maximum results. Although reducing sugar increases with days in most of the samples used, the rate of increase was higher in soybean than in cowpea where it even dropped in Day 3 (after rising) in two moderately resistant cowpea – IT 90K-277-2 and IT 95K-207-15.

Reducing sugar was higher in susceptible cowpea varieties than in the soybean varieties. This implies that insect infestation in cowpea will be higher than in soybean because insect do show preference for sugar which due to the instability of cowpea would be more readily available for them than in soybean.

CONCLUSION

Amylase activities and reducing sugars in soybean and cowpea are comparable.

In soybean, amylase activities began faster but not defined (scattered) in cowpea. Reducing sugar in soybean is more stable (and hence not available during insect pest infestation) while in cowpea it is not stable. Consequently, amylase activities and reducing sugars in soybean contributed significantly to making the crop resistant to insect infestation compared to cowpea.

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