CHANGES IN PROTEIN CONTENT AND ELECTROPHORETIC PATTERNS DURING GERMINATION OF *AMARANTHUS HYBRIDUS* SEEDS

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ABSTRACT

*Amaranthus hybridus* seeds were germinated in dark and light conditions over a 72-hour period. At specific intervals, the protein concentration and SDS – PAGE analysis of the seed protein extracts were determined. The results showed that there was a gradual decrease in the protein concentration from dry seeds to seeds germinated for 72 hours, with seeds germinating in the dark decreasing faster than such seeds germinated in the light. SDS – PAGE analysis further revealed that there were 3 major storage proteins in the dry seeds and these appeared in varying intensities over the 72-hour period. In some cases, the major protein totally disappeared as was the case of the protein with a molecular mass of 96.6 KDa which was actively utilized during germination especially up to the 48-hour period at which time the lowest possible number of protein bands occurred in both light and dark conditions.

Keywords: *Amaranthus hybridus*, Protein content, Germination, Electrophoretic patterns.

INTRODUCTION

Dry seeds are characterized by low metabolic rates, which may be due to their low moisture content of about 5 – 10%. However, the potential for metabolism still exists in dry seeds such that a lot of physiological and biochemical changes begin to occur when the apparent metabolic dormancy of dry seeds is disrupted by imbibition in the process of germination (Usha and Singh, 1996). The breakdown of stored proteins, lipids and carbohydrates occurs in the endosperm as soon as germination progresses in order for energy and other nutritional requirements to be provided for the embryo. Seed storage proteins are mobilized during germination and they function in supplying nitrogen and carbon to the growing embryo. This breakdown and mobilization of stored proteins affects the protein concentration in seeds during germination and may be reflected in the electrophoresis patterns in germinating seeds.

*Amaranthus hybridus* is a broad-leaved plant belonging to the family Amaranthaceae. It is usually cultivated as a pot-herb for medicinal purposes (Norman, 1992). It forms a large intake of leafy vegetable especially in West Africa (Ruskin, 1984). It grows vigorously, resists drought and pest and easily adapts to new environments. The seed has a protein content of about 16%, which compares favourably with conventional cereals such as wheat (12 – 14%), rice (7 – 10%) and maize (9 – 10%) (Harris *et al.*, 1980). Thus, it is one of the few non-grasses to produce significant amount of edible *cereal* grain. The seed head may be as long as 50cm resembling those of sorghum. Seeds are usually a little bigger than a mustard seed of about 0.9 – 1.7mm in diameter and varies in colour from golden, cream, pink, black or brown (Daloz, 1980). It is, therefore, a potentially important crop as it is used both as a leafy vegetable and cereal crop in the tropics. In Nigeria, it is being incorporated into the breeding programme for grain amaranth because it has the potential to impact early maturity from vegetable to grain types.

The aim of this research is to understand the phenomena that occurs in germinating *A. hybridus* seeds through an evaluation of the total protein content and study possible variations in the electrophoretic patterns during germination in light and dark conditions.

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Fig. 1: Protein Content in Seeds germinated in Light and Dark

![Graph showing protein content over time in light and dark conditions.]

- Dark
- Light

Fig. 2: Number of Protein Bands in Seeds germinated in Light and Dark

![Bar graph showing the number of protein bands at different times in light and dark conditions.]

- Dark
- Light

Fig 3 Schematic diagram of the protein bands under light and dark conditions.

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Key:
- L = Seeds germinated in Light
- D = Seeds germinated in Dark
- - = Lightly-Stained Bands
- = Medium-Stained Bands
- = Darkly-Stained Bands
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MATERIALS AND METHODS

A black seeded accession of *A. hybridus* was collected from the Nigerian Institute for Horticultural Research (NIHORT), Ibadan and used in this study. Seeds were germinated by weighing three grams each of seeds and placing them on moistened filter paper put in petri dishes. Germination was in light and dark conditions with various duration of time. Germination in continuous light was achieved by placing the petri dishes in a lit room with 40 watt fluorescent bulbs for the duration of the experiment. Germination in the dark was achieved by wrapping the petridishes securely with foil paper and keeping them inside a dark cupboard at room temperature (28 ± 2°C). Seeds were germinated for various length of time up to 72 hours before analysis. Dry seeds represented control samples. At each time point, seeds were extracted by grinding 2g samples in a mortar. Only those seeds that had imbibed water and showed visible differences in appearance with the aid of a magnifying lens from dry seeds were harvested and used for the analyses at each stage. One set of the experiment was used to determine the electrophoretic banding patterns using the SDS – polyacrylamide method as outlined by Odeighah and Osanyinpeju (1996). Electrophoretic mobility was calculated according to the formula of Weber and Osborn (1969) and molecular weights subsequently determined from a calibration curve using known mid-range molecular markers.

RESULTS

The proteins content in the seed extracts declined steadily from 227.5μg/ml in dry seeds to 45μg/ml after 72 hours of imbibition of water under both light and dark condition (Fig. 1). The rate of decline was however faster with seeds germinated in the dark compared to those germinated in light.

In the dry seeds a total number of 15 bands were resolved (Fig. 2). There was however variation in the number of bands resolved with time and condition of germination. Germination of *A. hybridus* seeds in light decreased the number of bands from 15 bands (mol. wt. ranging from 7.2 to 99.6 KDa) in dry seeds to 4 bands (mol. wt. 17 to 70.8 KDa) at 48 hours of germination, thereafter increasing to 20 bands (mol. wt. 14 KDa to 99.6 KDa) at 72 hours germination time. For seeds germinated under dark conditions the number of bands decreased from 15 in dry seeds to 13 (mol. wt. 9.0 to 97.4 KDa) by 72 hours of germination. There was no germination period (time) when the number of bands exceeded what was observed in dry seeds for seeds germinated in dark. However, the lowest number of bands, which was 7, was observed at 48 hours after germination and the molecular weight ranged between 10.0 to 68.7 KDa.

Different staining intensities were noticed for the bands resolved and a schematic representation is given in Fig. 3. Bands at 0.1cm and 5.2 cm were classified as major bands in the dry seeds as they were deeply stained and thick. The band at 0.1cm with a molecular weight of 99.6 KDa was completely degraded as soon as germination started and was not observed as a major band until 36 and 72 hours after germination in light conditions and at 60 hours in dark condition. The band at 2.1 cm (mol. wt. 65.0 KDa) appeared in majority of the germination periods both in light and dark conditions as major band close to the point where they occurred in dry seeds. It was however noticed that no bands was common to all levels of germination whether in light or dark conditions. However, some bands were common for both light and dark conditions at the same time of duration. For 6 hours, it was bands at 0.8, 1.8, 3.2 and 3.4 cm, while it was band at 2.3 cm only for 24 hours. No band was common to both light and dark conditions for 48 hours.
DISCUSSION

Seed storage proteins are usually considered to supply nitrogen and carbon to the growing embryo thus proteins are degraded during germination in order to provide amino-acids to developing seedlings (Marcellino et al., 2002). This was confirmed by the gradual reduction in protein concentration in the seeds. Decrease in total protein content with germination was also observed in winged bean (Usha and Singh, 1996). Sodium-dodecyl sulphate polyacrylamide gel electrophoresis of total proteins from dry seeds and germinating seeds of A. hybridus further revealed that different storage proteins exist in both light and dark conditions of growth. Although, some storage proteins were found to be common to both growth conditions i.e. light and dark for the same time period, in most cases the intensity and thickness of band varied. There was no storage protein that was common in both light and dark conditions over all germination periods.

One of the major components of the A. hybridus seed protein which was rapidly hydrolyzed during germination was the protein with a molecular mass of 99.6 KDa while the two major components with molecular masses of 65.0 and 7.2 KDa showed less degradation up to the 3rd day (72 hours) of germination. Thus, it may be opined that the protein with a molecular mass of 99.6 KDa is a major source of nitrogen needed to initiate germination especially during the first 2 days after which it gradually begins to reoccur and by the 3rd day after germination it has been reestablished. The other two major proteins of molecular masses of 65.0 and 7.2 KDa, however, do not seem to be required for the germination process.

Some storage proteins are rapidly degraded during germination serving as a source of nitrogen to germinating seeds; while some are not mobilized at all during germination and some new storage proteins are synthesized. In this paper, it was observed that as germination progressed there was loss of some storage protein especially up the 2nd day of germination, while several new polypeptide intermediates become visible at later stages of germination. This may imply that these proteins exists transiently in germinating seeds or that they do not really enhance the germination process. It also showed that the first 48 hours may be very critical in the germination of A. hybridus seeds as a lot of storage proteins are being actively utilized. The appearance of new intermediate proteins as germination progresses beyond the 2nd day suggest that such proteins may be needed to trigger the beginning of a synthetic activity in the germinating seeds thereby performing specific functions during germination (Hussain and Bushuk, 1991; Sun et al., 1978). Thus, the active synthesis and regulation of storage proteins in this study was manifested by the appearance and disappearance of protein bands.

CONCLUSION

In conclusion, the study has shown that while protein concentration may decrease as germination progresses, some specific storage proteins are of importance at the different stages of germination as shown by SDS – PAGE electrophoresis. It has also been shown that some storage proteins remain unchanged during germination. Further enquiry would be able to isolate and characterize such proteins and enzymes important at each germination period. This may become useful in the nutritional improvement of both the leafy and grain amaranth through genetic engineering.
REFERENCES


