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Review

Resistance of cowpea to Cowpea bruchid (*Callosobruchus maculatus* Fab.): Knowledge level on the genetic advances

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Cowpea [*Vigna unguiculata* (L.) Walp.] is an important food legume. This crop considered as source of dietary protein is also used as a leafy vegetable in many African countries. Its usage as food and animal fodder is focused on food security and diminishing malnutrition particularly in marginal areas. Bruchid (*Callosobruchus maculatus*) is the most damaging, cosmopolitan pest of stored cowpea grains especially in the tropical region. Damage caused by this pest in cowpea is irreversible, resulting in significant loss of the grains. Several management approaches including physical barriers and biological or chemical methods are used for controlling bruchid in cowpea. Considering the qualitative and quantitative damages caused by bruchid to cowpea in storage, it is important to tackle the bruchid infestation. Development of cowpea lines resistant to bruchid is the most effective, eco-friendly and durable approach to limit the losses associated with this pest. This paper presents a review of the importance of cowpea grain, the extent of bruchid damage in cowpea and the possible control measures. The advances of conventional and molecular breeding in building resistance against this cowpea pest in cowpea are also discussed highlighting the knowledge gaps and their implications. The knowledge of the status of genetic advances will inform breeders and researchers in the development of bruchid-resistant cowpea lines.

Key words: Bruchid resistance, cowpea, genetic improvement, storage insect pest.

INTRODUCTION

Ensuring global food security goal requires a range of strategies including the diversification and improvement of staple food crops and the reduction of post-harvest loss of foods. Cowpea [*Vigna unguiculata* (L.) Walp.], also known as black-eyed pea, is an important grain legume and staple food crop in many regions. Though the crop is native to Africa, its production areas include warm and hot regions of the globe including Asia, and South and Central America (Singh et al., 2003; Steele, 1976; Tan et al., 2012). Cowpea is the most economically important grain legumes of the Vigna genus belonging to the Phaseoleae tribe. Cowpea is a strategic crop that

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provides food and income, especially an inexpensive source of dietary protein to thousands of people in Africa and Asia (Gupta and Gopalakrishna, 2010). Cowpea is used as a natural complimentary food with cereals, due to its richness in lysine, an essential amino acid source which is low in most cereal grains (Gonçalves et al., 2016; Jayathilake et al., 2018).

Despite its importance as a food security crop, stored cowpea suffers damage by insects with bruchid (*C. maculatus*) being the most important post-harvest insect pest as reported in the tropics (Ndong et al., 2012). Bruchid infestation starts in the field when seed moisture content is still high and becomes more prevalent in storage (Ntoukam et al., 2000). Bruchid larvae is bore into the cowpea grains and eat it from the inside out. This feeding habit impairs not only the nutrients content of the grains but also its agronomic potential. Bruchid damage leads to seed yield loss in cowpea and in other legume crops during storage (Rees, 2004); it represents a serious threat for farmers traders and consumers, as it causes direct economic damage. This is due to food contamination with mycotoxins after a primary infestation and compromising the nutritional composition of the cowpea (Atanda et al., 2012). Therefore, the development of cowpea varieties resistant to bruchid is the best strategy to fight this store grain insect pest. In this review, we present the importance of cowpea, bruchid species description and damage, control measures against bruchid, status of knowledge on progress of molecular and conventional breeding for bruchid resistance in cowpea.

Utilization and nutritional quality of cowpea

Cowpea is the most used African leafy vegetables species (Singh et al., 2003). Different organs of cowpea plant including young leaves, fresh pods, fresh seeds and dry grains are consumed by humans and livestock because of their nutritional value (Boukar et al., 2015; Singh et al., 2003). The dry grain is the most important product which is eaten boiled, fried or steamed and found in a variety of food recipes such as salads, snacks and cakes (Singh et al., 2003). Cowpea is a highly nutritious crop (Sebetha et al., 2014; Tan et al., 2012). For instance, the dry grain is rich in proteins (23-32%), as well as essential amino acids such as lysine (427 mg.g−1 N) and tryptophan (68 $mg.g^{-1}$ N), (Singh, 2002; Timko et al., 2007). Cowpea dry grain is also rich in fiber and low in fat (Timko et al., 2007). Cowpea complements cereals well in terms of amino acids and, consequently, a diet combining both cereals and cowpea provides a balanced protein intake. The presence of minerals (iron and zinc) and vitamins (folic acid and vitamin B) in cowpea grains contributes to prevention of birth defects that arise during pregnancy (Diouf, 2011; Nielsen et al., 1993; Tan et al., 2012).

Beyond its importance for food and feed, the spreading indeterminate or semi-erect bushy cowpea provides ground cover, suppressing weeds and provides protection against soil erosion (Davis et al., 1991). The short growing period, drought tolerance aptitude and multi-purpose utilizations of the cowpea crop makes it an attractive alternative for farmers who cultivate in marginal, drought-prone areas with low rainfall and less developed irrigation systems, where infrastructure and malnutrition are major challenges. Cowpea cultivation is important because of its ability to improve soil fertility through atmospheric nitrogen fixation (Blade et al., 1997). It yields comparably high in harsh environments where other food legumes like soybean do not thrive (Shiringani and Shimelis, 2011).

Extent of bruchid damage in cowpea

Bruchid beetles are cosmopolitan pests of stored legume grains, including cowpea. They are widespread throughout the temperate and tropical regions of the world. Several species are agricultural pests and cause serious damage to stored grains. There are about 1300 species of grains beetles in the bruchidae family. Amongst these, 20 are identified as pests for stored legume grains especially in developing countries (Credland, 1994; Southgate, 1978). Credland (1994) reported four species that are of cosmopolitan importance; Acanthoscelides obtectus, *Callosobruchus maculatus*, *C. chinensis*, and *Zabrotes subfasciatus*. Southgate (1979) identified in addition to these four species, other species of Callosobruchus such as *C. rhodesianus, C. analis* and C. subinnotatus which also represent a group of storage insect pests. Cowpea bruchid (*C. maculatus* Fabricius) is the primary insect pest causing losses to stored cowpeas in the tropics (Olakojo et al., 2007). Bruchids lay eggs on the outside of cowpea grains which are clearly visible as small white dots on the grains. Bruchid damage on cowpea is directly related to the number of eggs deposited on seed surface in field or in storage and the larvae that hatch and bore into the seeds. Damage and weight loss in stored seeds are caused by larvae, which develop within the grains, consuming them from the inside out. Initial infestation of cowpea seeds occurs in the field just before harvest and the insects are carried into storage where population grows rapidly (Ntoukam et al., 2000). Cowpea bruchid causes significant loss and a total loss of the harvest may occur in absence of proper management of the pest population (Tarver et al., 2007). It has been reported that cowpea grains which are not stored with either chemical or non-chemical methods are often completely destroyed by bruchid in the first 10 to 12 months of storage (Umeozor, 2005). *C. maculatus* has caused huge weight loss, reduced viability and commercial value of cowpea seeds (Adedire et al., 2011). Damaged grains are full of

small holes and dead beetles may be found inside the grains (Tarver et al., 2007; Umeozor, 2005). These various damages can be quantitatively and qualitatively substantial, thus reducing the degree of usefulness and making the cowpea seeds unfit for human consumption as well as (Adedire et al., 2011; Singh et al., 2003). The various damages subjected to cowpea seed by bruchid results in an economic loss to farmers who are forced to sell their harvests as the pests prevent long-term storage and the possible benefit from the rise of price during shortage periods (Umeozor, 2005). These damages make bruchid species major economic pests in cowpea storage system, especially in developing countries. It is a serious agronomic constraint to cowpea production in sub-Saharan Africa, especially for many resource-poor farmers (Afun et al., 1991). This implies that the loss of cowpea production induced by bruchid has a negative effect on farmers' incomes as well on country richness. However, Ohiagu (1985) reported that 30 to 80% of total cowpea production, valued at over 300 million US dollars, is either lost or suffers damage annually due to the *C. maculatus* infestation on cowpea grains.

Control measures against bruchid

Cowpea is an important food crop in tropical countries, especially in West Africa (Adedire and Akinkurolere, 2005; Adedire et al., 2011). A high volume of cowpea production is lost due to damage caused by bruchid. The management of cowpea bruchid, together with proper cowpea cultivation systems, would help increase cowpea production. The core of any pest management is to reduce the biological fitness of the pest and to control its population (Cissokho et al., 2015). Farmers have employed several control measures for controlling bruchid and researchers have worked on some efficient methods for limiting damage of the storage pests. The control measures include, among others, storage hygiene, cultural, physical, biological, chemical control methods and use of inert materials (sand, stones, etc...). The chemical control appears to be the most used method, but it has harmful effects on man, living animals and environment (Adebowale and Adedire, 2006; Cissokho et al., 2015). For instance, phosphene fumigation or dusting with insecticides is the common practice to check bruchid infestation, but raises food safety health and environmental pollution concerns (Tripathy, 2016). Some authors have highlighted that use of chemical insecticides, besides the potential risks to human health and environment, is also not cost effective and can lead to a new resistance of pests (Akunne and Okonkwo, 2006; Ofuya et al., 2008; Thiaw and Sembène, 2010). Several authors have reported the use of biopesticides (plants extracts), especially those extracts from aromatics plants with repellent effects against *C. maculatus*. These include the use of soya oil, maize oil, banana plant juice and biocides (Kitamura et al., 1988); dried leaves of *Artemisia annua*, *Azadirachta indica* and *Ocimum gratissimum* (Brisibe et al., 2011); cashew balm based on *Anacardium occidentale* nut shell (Kpoviessi et al., 2017); *Vernonia amygdalina* leaves powder (Ileke, 2015); *A. indica* leaf powder (Akunne et al., 2013), etc. These various products act by suffocating the adult bruchid, restricting oviposition or even lead to the death of the insect. Bruchid pupation in Vigna seed usually ceases at temperatures below 20°C, and at storage temperature of 4-5°C the developmental stages following oviposition are drastically retarded (Tripathy, 2016). Besides, the work of Dent (2000) supported that the biological control measures including the use of pathogens, a range of invertebrate predators, parasites and parasitoids, have also been used to control bruchid species. These control measures have been used with varying degree of success in storage pest management systems. Dinarmus basalis has been used to control cowpea bruchid in storage systems in West Africa, reducing damage from 30 to 10% (Amevoin et al., 2007). In fact, some of the widely available products require expensive equipment and training for their use, and they are also associated with sanitary and environmental hazards. All these techniques, in spite of high efficacy are not completely effective; they are labor and resource intensive, and so not good for resource poor farmers (Isra et al., 2016). Therefore, development of cowpea varieties, leading to the breeding for bruchid resistance could be an ecofriendly and cost effective alternative. For developing countries or even countries in Australia, having access to conventional fumigants and chemical insecticides for management of bruchids is not simple. The success of applied methods has not always been established and work on bruchid control is still in progress (Credland, 1994; Somta et al., 2007).

GENETIC ADVANCES IN COWPEA IMPROVEMENT FOR RESISTANCE TO BRUCHID

Screening of cowpea lines for bruchid resistance

The insect origin and culture method used for the screening method all vary according to the authors. The insects are always reared on bruchid-susceptible varieties in the research center. Then, the insect culture is established in laboratory on bruchid-susceptible varieties to have the necessary population for a screening test (Lephale et al., 2012; Miesho et al., 2018; Mwila, 2013). Bruchid populations for the screening test are also obtained directly from market where the infested grains are collected. These bruchid colonies obtained from the market are used to establish the bruchid culture for the implementation of the screening test (Amusa et al., 2014; Lale and Kolo, 1998). The use of bruchid collected from the market infested seeds is the best way

Table 1. Day of infestation, number of bruchid and the cowpea seed amount according to the authors.

Amount of Cowpea seed	Number of bruchid individuals	Days of infestation	Authors
80 seeds	10 (5♂; 5♀)	7	Ojumoola and Adesiyun, 2014
5 g	7 (5♂; 2♀)	4	Lale and Kolo, 1998
10 g	5 (unsexed)	5	de Castro et al., 2013
10 seeds	4 (2♂; 2♀)	3	Amusa et al., 2013

♂ : Male ; ♀ : female

to have bruchid population for cowpea infestation in laboratory. Since the bruchid infestation starts in field and the insects are brought in the storage, the population of insect increases as well as the damage caused. Thus, in rural areas the bruchid species in storage are those from the field. The use of bruchid species collected from market-purchased cowpea in screening test allows ascertaining the real state of cowpea varieties used in screening process. To disinfest the cowpea grain in rural area, farmers usually use hermetic structures (Cissokho et al., 2015). These structures prevent the respiration of insect within the cowpea grains. The lack of respiration leads to the death of the insect. These structures are often used in rural area by farmers, to kill all insect stages; eggs, larvae, adult in grain. In research centers, the sterilization of the experimental cowpea grains from any previous stored-grain pests is done with oven at 30, 40, or 50°C for 24 h to kill any bruchid eggs or larvae that might be in the seeds (Amusa et al., 2013; Amusa et al., 2014; Lephale et al., 2012; Miesho et al., 2018). Other research showed experimentally that cowpea grains can be sterilized using cold temperature, indicating that low temperatures can kill the insect. Therefore, the sterilization of experimental cowpea grains can be done by maintaining the cowpea grains at cold temperature. These cowpea grains are usually maintained in a refrigerator at 17°C for five days (Mwila, 2013; Tefera et al., 2011) or at -20°C for 6-72 h (Appleby and Credland, 2003; Maro, 2017). Other reports have suggested that cowpea grains should be placed in plastic bags before storage in cold temperature (2-3°C) to control potential infestations brought from the field (de Castro et al., 2013). The latter method of sterilization aiming to use the plastic bags (hermetic structure) combined with cold storage is the best since it combines two modes of disinfestation. The infestation process during the screening test aims to put a number of individual insect per cowpea sample. The choice of the bruchid couple, the amount of cowpea seed and the mating day as suggested by other works are shown in Table 1.

The screening tests based on the use of equal number of cowpea seeds sample and on 2 couples of bruchid are better and more accurate than other methods. But we have to leave the insects on the seeds for more than 3 days of infestation and oviposition. To avoid crushing the eggs during manipulation and to insure hatching of the larvae, the insect should be left on cowpea samples for 5 to 7 days of mating and oviposition as suggested by other works (de Castro et al., 2013; Ojumoola and Adesiyun, 2014). These processes of screening are used in several studies to identify the most resistant cowpea variety to bruchid attack and damage. The sources of resistance to bruchid obtained in different studies are presented in Table 2.

Mechanisms of resistance to bruchid in cowpea germplasm

Bruchid resistance in cowpea results from a complex interaction between the host plant and bruchid species. This is a continuous process controlled by biochemical, physiological, and morphological features in the plants which can affect growth and development of the insects (Edwards and Singh, 2006; Lattanzio et al., 2005). Painter (1951) has categorized insect resistance into three mechanisms: non-preference, antibiosis and tolerance. The understandings of these mechanisms are essential for developing appropriate cowpea breeding strategies. Antixenosis (non-preference) resistance is the characteristic exerted by a host plant or seed to prevent the insect pest from using it for oviposition (egg-laying), feeding and shelter or all three (Dent, 2000). Other authors showed in their study that the damage caused by *C. maculatus* on cowpea grains is dependent on the plant genotype (Torres et al., 2016). In the study of Torres et al. (2016), it was evaluated the population of *C. maculatus reared* on beans from four cowpea cultivars. The result showed that lower cumulative emergence was found in the cultivars BRS Acauã and BRS Tapaihum and they showed an instantaneous rate of population growth compared with other cultivars. These cultivars' reactions toward *C. maculatus* indicated antixenosis resistance against this insect. Indeed, inoculation of BRS Acauã cultivar with the diazotrophic bacterial strain BR 3299 resulted in higher mortality of *C. maculatus* (Torres et al., 2016). The result of the screening of 50 cowpea genotypes resistance to *C. maculatus* (de Castro et al., 2013) showed that seven cowpea genotypes: IT85 F-2687, MN05-841 B-49, MNC99-508-1, MNC99-510-8, TVu 1593, Canapuzinho-1-2, and Sanzi Sambili exhibited non-preference-type resistance for the oviposition and feeding of *C. maculatus*.

Antibiosis is the mechanism of resistance where the

Table 2. Sources of resistance.

Cowpea variety	Reference
MNC99‑510‑8	de Castro et al., 2013
283	SECK, 1989
ALEGI × 5T	Miesho et al., 2018
MN05‑841 B‑49	de Castro et al., 2013
275	SECK, 1989
Tvu 11952	Singh et al., 1985
SEC1×SEC4	Miesho et al., 2018
IC107466	Tripathi et al., 2013
59-26	SECK, 1989
ACC2×ACC12	Miesho et al., 2018
Dan'ila	Lale and Kolo, 1998
Tvu 1593	de Castro et al., 2013
IT 845-2246-4	SECK, 1989
T189KD-391	Lale and Kolo, 1998
IT99K-429-2	IITA Germplasm
66-5	SECK, 1989
Kanannado	Lale and Kolo, 1998
IT81D-1032	Singh and Singh, 1990
NE4	Miesho et al., 2018
IT 85-2205	SECK, 1989
IT97K-1042-8	IITA Germplasm
WC16	Miesho et al., 2018
58-79	SECK, 1989
106817	Tripathi et al., 2013
IT81 D-1045	de Castro et al., 2013
NE39 × SEC4	Miesho et al., 2018
IT 81-1007	SECK, 1989
IT81D-1064	Singh and Singh, 1990
3B × 2W	Miesho et al., 2018
58-1GD	SECK, 1989
WC48	Miesho et al., 2018
IT81D-994	Norris, 1996
IT85 F-2687	de Castro et al., 2013
182	Miesho et al., 2018
Sanzi Sambili	de Castro et al., 2013
2419	Miesho et al., 2018
KVX 30-G246-2-5K	SECK, 1989
Canapuzinho-1-2	de Castro et al., 201 3
WC42	Miesho et al., 2018
IT97K-499-8	IITA Germplasm
Tvu 2027	Miesho et al., 2018; Singh et al., 1985
MNC99-508-1	de Castro et al., 2013
106816	Tripathi et al., 2013
ACC23 × 3B	Miesho et al., 2018
Tvu 11953	Singh et al., 1985
106037	Tripathi et al., 2013
58-162	SECK, 1989
WC67	Miesho et al., 2018
106812	Tripathi et al., 2013
IT81 D-1045 Ereto	de Castro et al., 2013
IT90K-76	Miesho et al., 2018; Singh, 2002

Table 2. Sources of resistance.

311138	Tripathi et al., 2013
IT97K-499-35	Miesho et al., 2018; Singh, 2005
108749	Tripathi et al., 2013
Pusa Komal	Tripathi et al., 2015
IT84S-2246-4	Miesho et al., 2018; Singh and Singh, 1990
328859	Tripathi et al., 2013
IT95K-207-15	Miesho et al., 2018; Singh, 2002
IC328859	Tripathi et al., 2015
D2A2	SECK, 1989
381583	Tripathi et al., 2013
IT97K-1042	IITA Germplasm
106815	Tripathi et al., 2013
Red caloona	Lephale et al., 2012

colonized seeds affect the insect's nutrition, development, reproduction and survival (Dent, 2000). According to Painter (1951), when antibiosis is in effect, the insect pest performance will be low, the reproduction potential of the host will be reduced, the rate of development of the insect is slow or the host will injure or kill the insect pest or indirectly affect the insect pest depending on the time of exposure (Painter, 1951). Allelochemical substances and primary metabolites (phyto-toxins) are generally associated with antibiosis mechanisms. Several studies have shown that hydoxycinnamic acids (phenolics) are important in grain resistance to storage pests. The evaluation of 50 cowpea genotypes resistance to *C. maculatus* studied by de Castro et al. (2013) for the preference for oviposition and the development of bruchid showed that only two cowpea genotypes: IT81 D-1045 Ereto and IT81 D-1045 Enramador exhibited antibiosis-type resistance to *C. maculatus*.

Tolerance is the ability of the host plant to resist the action of a pest, to survive infection, to rapidly recover, repair, withdraw effect or withstand infestation (Dent, 2000). Tolerance denotes a mere biological relationship between the host organism and the pest, while antibiosis and antixenosis (non-preference) are chemicals and physical resistance devices of the host (Beck, 1965). Horber (1989) reported that host plant tolerance mechanisms were inapplicable in the case of storage pests, because damage inflicted on stored produce is irreversible, since in storage the seed is not connected to the plant. Therefore, only antibiosis and antixenoxis (non-preference) mechanisms of resistance are relevant in the study of cowpea resistance to bruchid. It has been reported that the three mechanisms of resistance, wherever applicable, will influence the population dynamics of insects either under laboratory (storage) or field conditions by their action on the life history parameters: initial colony size, developmental period, fecundity of adults, and mortality of larvae or adults (Dent, 2000).

In other reports, it has been reported a high antinutrient levels, mainly antitryptic and antiamylasic activity in bruchid resistant cowpea lines (Piergiovanni et al., 1994). The results of the experiment showed that a high activity of a single inhibitor class (porcine amylase, bacillus amylase, bovine chymotrypsin and trypsin) was typical of the bruchid susceptible lines, leading to the conclusion that breeding for high protein inhibitor content could be an effective way of obtaining cowpea lines improved for the naturally resistance to storage pest attack.

In Uganda, Miesho et al. (2017) investigated the roles of seed coat and cotyledon in cowpea seeds resistance to bruchid. They found that compounds in the seed coat (tannins, flavonoids, total phenolic content and anti-oxidant activity) and in the cotyledons (carbohydrates, proteins, and α-amylase inhibitory activity) conferred resistance to bruchid infestation (Miesho et al., 2017). Seeds of wild species (Vigna luteola and Vigna vexillata) and varieties of Vigna were studied for their seeds coat tannins and their resistance to bruchid. For instance, tannins can deter poison or starve bruchid larvae that feed on cowpea seeds (Lattanzio et al., 2005). It has been shown that the cultivar TVu 2027 is moderately resistant among screened cowpea accessions (Lattanzio et al., 2005). In addition, two susceptible cultivated accessions; Vita 7 and IT 84E-1-108 showed no α-amylase inhibitory activity in the cotyledons of undamaged Vita 7 seeds while, within the seed coat, the tannin content was found to be thirteen times higher in undamaged Vita 7 seeds compared to the IT 84E-1-108 infested seeds content. It has been reported that seeds of the common bean are resistant to Adzuki bean weevil largely because of the presence of α-amylase inhibitor (αAI-1), and seed protein that is toxic to the larvae (Shade et al., 1994). To control αAI-1 tolerant bruchid species such as A. obtectus and to avoid the development of resistance to αAI-1, varieties carrying this transgene should be protected with additional control measures in order to strengthen the crops resistant to bruchid species (Shade et al., 1994). Other work showed

that the α-amylase inhibitor (αAI-1) present in seeds of transgenic chickpea and cowpea lines significantly increases their resistance to two important bruchid pest species (*C. chinensis* and *C. maculatus*) (Lüthi et al., 2013). In Nigeria, the role of chemical factors of cowpea seed coat in the resistance of cowpea varieties to bruchid, has been investigated under laboratory conditions (30-35°C and 65-67% RH) (Lale and Makoshi, 2000). There are significantly greater numbers of eggs laid on de-coated as opposed to intact Kanannado seeds whereas significantly fewer eggs were laid on de-coated than on intact IT89KD-391 or Borno brown seeds. Egg-hatch was reduced in seeds with intact seed coats by 88.6%, while the proportion of eggs that failed to hatch in de-coated seeds was 31.9% (Lale and Makoshi, 2000). Chen et al. (2002) studied the insecticidal activity against bruchid exhibited by defensin encoded by mungbean cDNA. They reported that a cDNA encoding VrCRP (small cysteine-rich protein) was the first reported plant defensin which exhibited *in vitro* insecticidal activity against *C. chinensis*. Moreover, during this study, the artificial seeds containing 0.2% (w/w) of the purified VrCRP-TSP were lethal against larvae of the bruchid *C. chinensis* (Chen et al., 2002).

Breeding for bruchid resistance in cowpea

Plant breeding refers to the genetic alteration of plants to satisfy human needs (Falconer and Mackay, 1996; Sansern et al., 2010). Various mating designs and arrangements help breeders generate information to understand the genetics of a trait of interest and also to define a base population to begin within a breeding program (Acquaah, 2012). Knowledge on the genetics of bruchid resistance is necessary to understand the mechanisms of gene action controlling the resistance of cowpea bruchid among the available cowpea varieties. This knowledge will be useful for effective selection gain in the breeding program focusing on the resistance trait in cowpea. Inheritance patterns for bruchid resistance in cowpea seed are complicated as the seed components have different ploidy levels (Chen et al., 2007; Somta et al., 2006a). Within the seed, the embryo and endosperm belong to progeny tissues and are diploid and triploid, respectively while seed coat is derived from maternal tissue. Consequently, the genetic control of resistance to storage insects may range from monogenic to oligogenic (Chen et al., 2007; Somta et al., 2006a).

Breeding to combine seed and pod resistance has been explored to reduce losses associated with bruchid infestation in cowpea (Ntoukam et al., 2000). IITA has developed high-yielding varieties for both sole and intercropping, with resistances to major insect pests, diseases, nematodes and parasitic weeds. For example, IT84S-2246-4 was reported to be one of the superior lines which, in addition to its resistance to bruchid, combines resistance to other pests such as aphids, thrips and ten other diseases (Singh and Singh, 1990). Two high yielding and bruchid resistant cowpea lines (LORI NIEBE and CRSP NIEBE) were developed and tested in IITA Breeding Program (Ntoukam et al., 2000). Most of the improved varieties are obtained by crossing bruchid resistance sources with those susceptible but with desirable characteristics to spread bruchid-resistance within other varieties. Several bruchid-resistance cowpea lines have been developed using resistance genes from TVu-2027 and the varieties have been released to farmers in many countries (Singh, 2005; Singh et al., 1996). TVu-2027 is the single source of bruchid resistance, so there are reasons to believe that bruchid could rapidly evolve to break the resistance. According to Shade et al. (1996), after selection on resistant cowpea seeds for over 53 generations, C. maculatus was able to develop a new biotype to overcome Tvu-2027. Therefore, for durable insect-resistance, new sources of resistance are necessary for developing multiple resistance lines. Many sources of resistance have been identified (Table 2). These sources of resistance have been identified as resistant among local varieties, by screening local cowpea varieties against cowpea bruchid infestation (*C. maculatus*) (Lephale et al., 2012; Miesho et al., 2018; Mogbo et al., 2014).

Tripathy (2016) highlighted that screening of primary, secondary or tertiary gene pools from local sources, introduction and acquisition of germplasm from exotic sources and recombinants resulting from crossings of selected parents from different sources may pave the way for identification of news bruchid resistant genotypes. Inter-specific crossing barriers between gene pools often limit the transfer of resistance gene (available in wild species) to cultivated varieties (Shaheen et al., 2006). The inter-specific crossing barriers such as failure of seed setting can be overcome by the use of embryo rescue culture techniques. Nevertheless, undesirable characters are co-inherited during interspecific crosses. Consequently, breeding for resistance against storage insect pests using wild relatives as gene sources may improve resistance but often reduces the quality of the product or may even make it unfit for consumption (Shaheen et al., 2006). Breeding programs may be based continually on use of well-adapted and desirable varieties and resistant varieties (wild species) for transferring desirable traits to the cultivated cowpea. These sources of insect-resistant traits must continue to be identified in cowpea germplasm. Additional tools involving molecular approaches must be integrated to determine molecular markers linked to bruchid resistance in cowpea. Subsequently, these will be useful as it helps to make available news insect-resistant cowpea varieties.

Molecular marker technologies in improving cowpea for resistance to bruchid

Bruchid resistance in cowpea is a complex trait controlled

by a few major genes (Tripathy, 2016). This can slow the introgression of the trait into elite lines. In the last decades, progress in molecular genetics has provided breeders with powerful molecular genetic tools such as linkage maps and quantitative trait loci for fast tracking of a specific trait of interest. The microsatellites or Simple Sequence Repeats (SSR) marker Vm50 was found to be closely associated with the delay in emergence of *C. maculatus*, explaining 20% of the variation (Fatokun, 2000). Some efforts need to be furnished to the development of improved cowpea varieties with bruchid resistant traits. Following similar studies on identification of quantitative trait loci for bruchid resistance in others legume crops (Somta et al., 2007; Somta et al., 2006a; Souframanien et al., 2010), the development of cowpea resistant to bruchid will likely be based on the identification of quantitative trait loci (QTL) associated to the bruchid-resistant traits. Besides, there exists a great diversity of resistance depending upon legumes crop and bruchid species. Souframanien et al. (2010) have carried out a study on the identification of QTL for bruchid resistance in black gram using a Recombinant Inbred Lines (RIL) population derived from interspecific cross of *V. mungo* var. mungo (cv. TU 94-2, bruchid susceptible) and *V. mungo* var. silvestris (bruchid resistant). In this study, Souframanien et al. (2010) generated a linkage map using a 104 line RIL population in the F9 generation with 428 markers. The RILs used in this study exhibited 0–100% resistance, which is a high level of variation in percentage adult emergence and (0-105 days) for the developmental period. Moreover, two QTLs (Cmrae1.1; Cmrae1.2), have been identified for the percentage adult emergence; on linkage group (LG) 3 and 4, respectively. Finally, six QTLs were identified, with two QTLs (Cmrdp1.1 and Cmrdp1.2) on LG 1, three QTLs (Cmrdp1.3, Cmrdp1.4, and Cmrdp1.5) on LG 2, and one QTL (Cmrdp1.6) on LG 10, for developmental period,

The inheritance of seed resistance to bruchid in cultivated mungbean has also been studied . In this study, the authors carried out quantitative trait loci (QTL) analyses for resistance to *C. chinensis* and *C. maculatus* using F2 (*V. nepalensis* × *V. angularis*) and BC1F1 [(*V. nepalensis* × *V. angularis*) × *V. angularis*] population. The populations generated from crosses between *V. nepalensis* and *V. angularis,* the bruchid resistant species and the bruchid susceptible species respectively were used. From both populations, the report identified seven QTLs for bruchid resistance, including five QTLs and two QTLs for resistance to *C. chinensis* and *C. maculatus* respectively. Out of five QTLs for resistance to *C. chinensis*; two QTLs, one on LG1 and another on LG2 were colocalized with seed size QTLs indicating that increase in seed size was associated with susceptibility to *C. chinensis*. The QTL for bruchid resistance has been mapped (Somta et al., 2006b). These QTLs are near 82.40 cM and 75.04 cM for LG1 and LG2 respectively (Muñoz-Amatriaín et al., 2017). Within a population derived from rice bean (*V. umbellata*-resistant parent) × (*V. nakashimae*-susceptible parent), a mapping study revealed that bruchid resistance in rice bean is controlled by 4 QTLs (Somta et al., 2006b). It has been co-localized; two QTLs in the same LG1 and/ or responsible for resistance to both *C. maculatus* and *C. chinensis* while the other two express differential effects on Callosobruchus species.

PERSPECTIVE

The development of cowpea varieties effectively resistant to bruchid requires the availability of genomic resources for cowpea. Integration of desired traits from different backgrounds of cowpea has led to the development of cowpea gene pools and the development of improved cowpea varieties suitable for different agro-climatic conditions. Conventional breeding in conjunction with genetic tools like molecular markers could boost the breeding process. The use of wild cowpea species to transfer the bruchid-resistant traits in common cowpea varieties must pave the way for an efficient development process for cowpea resistant to bruchid. When a preferred genotype is not available or in the case of narrow genetic base in the primary gene pool, plant breeders often resort to tap genetic variability from allied species through wide hybridization or create novel plant types through mutagenesis and genetic transformation (Tripathy, 2016). However, the successes of this genetic transformation take into account potential adoption or farmer's acceptance which is related to the clarification of safety for human consumption and other kinds of utilization.

Molecular breeding approaches have been initiated in some cowpea breeding programs using LGC Genomics, which converted about 1100 mapped SNPs for use with the KASP platform (Boukar et al., 2016). In similar study, the frequency and positional distribution of genetic diversity in the cowpea genetic map have been investigated in distribution of genetic variation based on the anchoring of 25,537 WGS scaffolds using mapped iSelect SNPs (Muñoz-Amatriaín et al., 2017). The result revealed that nearly half of the 1,036,981 SNPs were discovered from the 36 diverse cowpea accessions which were anchored on the genetic map. This information was used to identify the SNP frequency and distribution across the eleven cowpea LGs used for this study (Muñoz-Amatriaín et al., 2017). Nevertheless, efforts must be continued to generate more DNA-markers, which will help to identify new genes for resistance to major biotic stresses including insect-resistance as well as abiotic stresses. These will represent good tools for breeding applications. A number of scientific advances in cowpea genetic linkage maps, and QTL associated with some desirable traits such as resistance to Striga, bacterial blight, Fusarium wilt, Macrophomina, root knot

nematodes, aphids, foliar thrips and QTL controlling domestication-related traits in cowpea (Boukar et al., 2016; Lo et al., 2018; Tripathy, 2016). Efforts must be continued to highlight QTL associated with bruchid (*C. maculatus*) resistance in cowpea, which will be a breeding tool for cowpea breeders.

CONCLUSION

In tropical and subtropical regions, cowpea is the cheap source of proteins. This crop can save the under-nourished population from malnutrition in developing countries. But, its production and productivity is always limited by pests attack in field as well as in storage. In storage the damage is essentially caused by *C. maculatus* which is the main postharvest pest of cowpea. Control strategies such as biological, chemical, cultural and mechanical methods, may not adequately address the problem of bruchid damage. Consequently, genetic improvement of cowpea for bruchid resistance is by far the most cost effective and long-term measure to limit the damage of this pest. However, the genetic development of cowpea varieties for bruchid resistance might not be achieved due to linkage drag, biotype variation, lack of interspecific compatibility and narrow genetic base in the gene pools. Moreover, the sources of resistance are very few among the large cultivated varieties, while wild species of cowpea are known to have multiple resistance mechanisms against bruchid. Unfortunately, these latest varieties of cowpea are non-useful and are unfit for consumption.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Germplasm collection and morphological characterization of local accessions of tigernut (*Cyperus esculentus* L.) in Ghana for conservation and utilization

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Tigernut (*Cyperus esculentus* L.) is a nutritious crop of the sedge family. In Ghana available local accessions have not been collected and characterized for conservation and utilization purposes. The objective of this study was to collect, conserve and characterize twenty-four local accessions of tigernut in Ghana based on agro-morphological traits. The ANOVA revealed significant ($p<0.05$) differences among the accessions for all the traits studied except for hundred nut weight, indicating the presence of sufficient variability among the accessions. The hierarchical cluster analysis put the accessions into six major groups confirming a wide range of diversity among the accessions. The biplot of the principal components analysis revealed the scattering of the accessions in all the quarters which further suggest a higher level of variability among the accessions studied. The PCA also revealed that the first five PC accounted for a total of 88.4% variability among the accessions. PC1 accounted for 45.6% of the total variation with an Eigenvalue of 6.84. The correlation analysis among the traits showed significant and positive correlation between number of nuts and good nuts (r=0.94) and detached nuts and attached nuts. However, there was significant negative correlation among nut width and detached nuts (r = - 0.88) and harvest index and biological yields (r = - 0.77). Based on the study, accessions TPY, CCB, BB, DY, ADL, KB, KAY, WY1 and BKB which recorded high values for number of nuts, good nuts, nut length, nut width and harvest index could be included in breeding programs for varietal development of tigernut in Ghana.

Key words: Tigernut, *Cyperus esculentus*, Ghana, morphological characterization, cluster analysis, germplasm, principal component analysis, correlation, variability.

INTRODUCTION

Tigernut belongs to the family *Cyperaceae*, and produces rhizomes from the base and tubers that are somewhat spherical (Cortés et al., 2005). The plant is not really a nut but a tuber which was first discovered some 4000

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years ago (Lowe et al., 2000). It has other names like yellow nutsedge, chufa, flat sedge, rush nut, water grass, earth almond, northern nut grass and nut grass (Shilenko et al., 1979). The tubers are very nutritious, typically, hundred grams of the nuts contain 386 kcal (1635 kj) of energy, 7% proteins, 26% fats (oils), 31% starch, 21% glucose and 26% fibre of which 14% is non-soluble and 12% soluble (Burden, 2003). It also contains vitamins A, B1, D2 and E, while the minerals include, Calcium, Magnesium, Sodium, Potassium, Copper, Iron and other beneficial enzymes (Burden, 2003). Tigernut is used as a source of food, medicine and perfumes (De Vries, 1991). It can be eaten raw, roasted, dried, baked or made into a refreshing beverage called Horchata De Chufas or tigernut milk which is very nutritive.

Medically, the nuts are reported to be aphrodisiac, carminative, diuretic, stimulant and tonic which can be used in the treatment of constipation, high blood pressure and diarrhoea (Oladele and Aina, 2007). Economically tigernuts provides Ghana with foreign exchange through its exportation. In 2010 Ghana exported 63,462 tonnes of tigernut valued at US$ 25,130.82 to countries such as England, Japan and America (GEPC, 2010). Its cultivation also provides jobs to about 85% of the youth and women in the major growing areas of Ghana (Tetteh and Ofori, 1998). In spite of the nutritional, medicinal and economic value of tigernut, the crop still remains an orphan.

Research into the production and general improvement of tigernut through breeding has received very little attention and farmers still cultivate landraces which are low yielding and susceptible to diseases and pest. Available accessions of tigernut in Ghana have not yet been collected, characterized and conserved. This further exposes available landraces to erosion of their genetic resources and limits breeding of improved varieties. Germplasm characterization plays an important role in varietal development of crops as genotypes with desirable traits are identified and utilized in the crop improvement programmes. Knowledge on the genetic diversity and variation among available accessions is very important for pragmatic use of plant genetic resources and also to determine evolutionary relationships (Zada et al., 2013). It will also aid in the early identification and exploitation of desirable traits such as high yield and early maturity. The existence of genetic variation among accessions can be employed as the basis for improving yield and other potentials of crop plants (Makinde and Ariyo, 2013).

Morphological attributes of crops have been employed as characterization tools among crops such as clusterbean (Manivannan et al., 2016), groundnut (Makinde and Ariyo, 2013), cowpea (Manggoel et al., 2012). Morphological traits has also been used to determine the extent of genetic variation among purple and yellow nut sedge accessions (Cruz and Baltazar, 2001, Peña-Fronteras et al., 2009, Okoli et al., 1997, Casimero et al., 1999). Before any effective work can be done on tigernut, there is the need to collect and characterize the local accessions that are available in Ghana. The objective of this study was to collect and characterize the accessions of tigernuts available in Ghana to promote their conservation and utilization.

MATERIALS AND METHODS

Study area

The study was conducted at the multipurpose nursery of the College of Agriculture, University of Education, Winneba Mampong-Ashanti, during the minor season growing season. Mampong-Ashanti lies within longitude 0°05"W and 1°30"W and latitude 6°55"N and 7°30"N and altitude 395 m above sea level. The area has an average annual rainfall of 1270 mm in two seasons (March and September) and a mean daily temperature of 27°C (Metrological Service, Mampong, 2010).

Germplasm collection

Twenty-four accessions of tigernut were collected from six major tigernut growing regions in Ghana that is Eastern region (Asukese Donkokrom, Nkwakwa), Volta region (Krachi), Upper East region (Bawku), Upper West region (Wa), Central region (Kasoa, Badwiase, Gomoa Feteh, Twifo Praso) and Brong Ahafo (Techiman). Table 1 shows the names, colour and collection area of the accessions. The accessions collected were kept in polyethylene bags and tagged with their names. The accessions were named using the first letters of the towns where they were collected and the colour of the nuts. Numbers were used to differentiate accessions from the same town which were having the same colour for example WY1 meaning Wa Yellow, first accession. Figure 1 shows the map of Ghana showing the location of regions and the towns where the accessions were collected. Figure 4 shows the pictures of some of the accessions collected.

Germplasm evaluation

The accessions were evaluated using RCBD with five replications in plastic buckets. The volume of the bucket was 12212 cm^3 and was fully filled with heat sterilized sandy loam soil. The buckets were arranged 50 cm within rows and 100 cm between rows. Each bucket contains five stands of tigernut per genotype. The five stands were arranged 5 cm within rows and 5 cm between rows in the buckets. The plants were raised under irrigation and manual weeding was done regularly in the buckets as well as between and within the rows of the arranged buckets. Data was collected on all the five stands in the buckets. Data collection was started when the plants were a week old. There was no fertilizer and pesticide application. The following traits were evaluated; percentage germination, number of tillers/stand, number of attached nuts/stand, number of detached nuts / stand, number of good nuts/stand, number of bad nuts /stand, total nuts /stand, number of leaves/ plant, number of ridges /nuts, nut length and width (cm), hundred nut weight, economic yield, biological yield and Harvest index (%).

Statistical analysis

The data collected was subjected to Analysis of Variance (ANOVA) using the GenStat statistical software, version 11.1 (GenStat, 2008).

Dissimilarity matrix based on Euclidean distance was estimated using GenStat 11.1 version. The scores of the dissimilarity matrix

Table 1. Source and colour of accessions collected.

S/N	Accessions	Collection place/area	Region	Colour
1	ADS	Asukese Donkorkrom	Eastern	Yellow
2	KB	Krachi	Volta	Black
3	KY	Krachi	Volta	Yellow
4	KAB	Kwanyako	Central	Black
5	KAY	Kwanyako	Central	Yellow
6	WY 1	Wa	Upper West	Yellow
7	DY	Bodwiase	Central	Yellow
8	BB	Bawku	Upper East	Black
9	BY	Bawku	Upper East	Yellow
10	TY	Techiman	Brong Ahafo	Yellow
11	BLB	Badwiase	Central	Black
12	BLY	Badwiase	Central	Yellow
13	CCB	Kasoa	Central	Black
14	CCY	Kasoa	Central	Yellow
15	AY	Nkwakwa	Eastern	Yellow
16	TPB	Twifo Praso	Central	Black
17	TPY	Twifo Praso	Central	Yellow
18	BKB	Badwiase	Central	Black
19	WY2	Wa	Upper West	Yellow
20	BKY	Badwiase	Central	Yellow
21	WB	Wa	Upper West	Black
22	GFB	Gomoa Fetteh	Central	Black
23	GFY	Gomoa Fetteh	Central	Yellow
24	ADL	Asukese donkokrom	Eastern	Yellow

ADS (Asukese Donkorkrom Short), KB (Krachi Black), KY (Krachi Yellow), KAB (Kwanyaako Asamoahkrom Black), KAY (Kwanyaako) Asamoahkrom Yellow), WY1 (Waa Yellow 1), DY (Danso Yellow), BB (Bawku Black), BY (Bawku Yellow), TY (Techiman Yellow), BLB (Badwiase Local Black), BLY (Badwiase Local Yellow), CCB (Cape Coast Black), CCY (Cape Coast Yellow), AY (Aduamoah Yellow), TPB (Twifo Praso Black), TPY (Twifo Praso Yellow), WY2 (Waa Yellow 2),BKB (Bawjiase Kwahu Black), BKY (Bawjiase Kwahu Yellow), WB (Waa Black), GFB (Gommoa Fetteh Black), GFY (Gommoa Fetteh Yellow), ADL (Asukese Donkokrom Short).

were used to perform a hierarchical cluster analysis (Ward, 1963). Principal Component Analysis (PCA) based on the traits was performed to find out the relative contribution of the different traits to the total variation in tigernut. A biplot was drawn to show the relationship between the accessions and the traits using the Eigen values associated with the components versus the number of the component. Pearson (1901) Correlation coefficients was carried out for all the traits and a correlation matrix was prepared to understand the relationship among the different traits.

RESULTS

Variation in agronomic traits among the accessions

The Analysis of Variance (ANOVA) and its corresponding coefficient of variation (Table 2) revealed significant differences ($p<0.05$) among the accessions for all the traits studied except for hundred nut weight. Percentage germination ranged from 43.0 to 88.0% with accession KY recording the highest germination percentage and ADS recording the least. Accession CCB had the highest number of tillers per stand which ranged from 2.6 to 4.6 with accession GFY having the least number. For number of attached nuts per stand, accession BB showed the highest value with GFY recording the least value. The number of detached nuts per stand ranged from 3.80 to 22.0, accession BB was observed to have the highest and CCB had the least. Accessions CCB, KY and DY recorded the same number of bad nuts per stand of 5.6 which was the highest and accession BB had the least number of bad nuts per stand of 1.6. It can be observed in Table 6 that accession BB had a highest good number of nuts per stand (42), while ADL the lowest (4.80). For total number of nuts per stand Accession BB had highest number (43.0) while accession GFY had the least number (6.20). Accession GFY was observed to have the highest number of leaves per plant and accession DY the least. The Table clearly shows that, accession ADL had the longest nut length of 1.94cm among the accessions studied while accession BB had the shortest nut length of 0.70cm. Among the accessions studied, ADL demonstrated the highest hundred seed weight of 295.3g and accessions BB demonstrated the lowest of 17.8g.

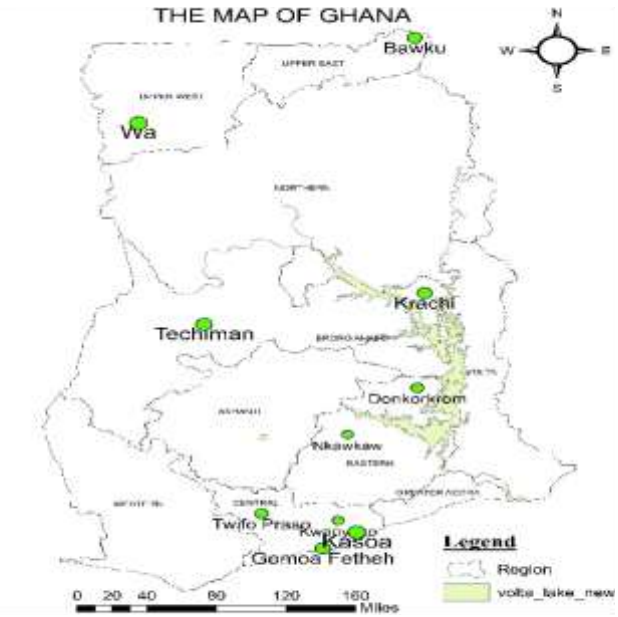

Figure 1. Map of Ghana showing the location of regions and the towns where the accessions were collected.

Table 2. Mean and the standard error of the agronomic traits for the accessions evaluated.

Accession	%Gem	NTS	ANS	DNS	BNS	GNS	TNS
ADS	43.00±2.55	3.40±0.24	7.80±0.97	10.20±1.11	4.40±0.40	15.00±1.58	18.20±1.62
KB	81.00±7.48	3.00±0.00	6.80±0.96	5.00±1.09	4.20±0.48	8.00±0.94	11.60±1.56
KY	88.00±6.44	2.80±0.20	4.60±0.40	9.00±1.34	5.60±0.60	9.20±1.20	12.40±1.56
KAB	73.00±5.15	2.80±0.20	5.40±0.67	4.20±0.58	2.80±0.58	9.20±0.97	13.40±1.69
KAY	83.00±7.18	2.60±0.24	4.60±0.81	7.20±0.91	4.80±0.80	8.20±0.86	15.00±1.51
WY	77.00±5.12	4.20±0.37	5.20±1.15	9.00±1.64	4.60±0.40	10.80±1.02	12.80±1.49
DY	48.00±8.00	4.00±0.45	11.80±1.06	12.60±1.69	5.60±0.74	17.40±1.66	22.00±1.04
BB	48.00±8.00	4.20±0.37	26.00±3.61	22.00±1.76	1.60±0.50	42.00±5.02	43.20±6.22
BY	48.00±6.44	3.40±0.25	8.60±1.02	19.20±2.85	3.80±0.48	2.60±3.31	26.20±3.07
TY	62.00±7.35	2.80±0.37	5.00±1.04	8.20±1.02	3.60±0.67	9.80±1.82	13.00±1.09
BLB	69.00±5.34	3.60±0.25	8.20±0.66	5.00±0.31	4.20±0.73	10.00± 1.89	12.20±2.13
BLY	68.00±8.46	2.80±0.37	4.80±0.96	8.00±1.37	5.40±0.74	8.60±1.03	12.40±1.50
CCB	75.00±5.70	4.60±0.40	7.60±0.50	3.80±0.58	5.60±1.16	8.00±0.83	12.00±0.00
CCY	81.00±2.92	3.00±0.55	4.00±0.44	8.40±1.93	2.80±0.48	8.60±1.20	10.40±1.16
AY	75.00±4.18	3.40±0.25	3.40±1.16	8.20±0.80	2.40±0.50	13.80±1.15	13.20±1.06
TPB	87.00±3.00	3.40±0.25	8.40±1.32	8.20±1.39	3.00±0.54	15.40±1.28	13.00±1.67
TPY	78.00±4.06	3.40±0.25	3.20±0.20	10.20±0.97	3.00±0.70	9.60±0.81	11.20±1.31
WY2	74.00±7.97	3.20±0.20	3.00±0.54	8.80±1.59	2.40±0.50	9.40±1.80	14.80±1.59
BKB	77.00±5.15	3.20±0.20	8.00±0.83	4.40±0.24	4.80±0.37	9.40±0.67	11.20±0.73
BKY	63.00±4.64	3.00±0.00	4.20±0.66	8.00±1.64	1.80±0.20	9.00±0.89	10.60±1.12
WB	65.00±4.47	2.80±0.37	6.60±0.97	5.40±1.07	2.00±0.54	10.00±1.14	11.80±0.97
GFB	80.00±5.00	3.20±0.20	6.60±0.81	6.00±0.83	4.00±0.44	8.80±0.73	12.20±0.97
GFY	66.00±4.85	2.40±0.25	1.80±0.20	4.60±0.40	2.00±0.44	4.80±0.37	6.20±0.73
ADL	50.00±6.52	3.00±0.32	2.20±0.20	6.00±0.63	1.80±0.37	7.60±0.81	8.80±0.80
LSD (5%)	16.03	0.85	0.43	3.04	3.18	1.58	5.14
CV%	18.50	20.70	20.70	36.90	30.20	35.00	28.20

%Gem= percentage germination, NTS=number of tillers /stand, ANS= number of attached nuts / stand, DNS= number of detached nuts /stand, BNS= number of bad nuts / stand, GNS= number of good nuts /stand, TNS= total number of nuts / stand.

Table 2. Contd.

Accession	NL	NW	NRN	100SW	EY	BY	HI
ADS	1.38±0.06	1.12±0.05	3.00±0.0	90.7±4.69	1.91±0.34	5.27±0.51	35.34±4.26
KB	1.68±0.06	1.32±0.05	3.80±0.20	129.0±7.55	1.70±0.11	5.14±0.37	33.89±3.67
KY	1.14±0.02	1.14±0.06	3.00±0.00	76.1±4.50	2.52±0.10	4.55±0.19	55.59±1.40
KAB	1.52±0.05	1.24±0.05	3.80±0.20	113.7±6.76	2.55±0.13	3.77±0.17	67.74±0.72
KAY	1.58±0.13	1.02±0.03	3.00±0.0	89.7±6.20	2.72±0.30	4.76±0.55	57.56±2.89
WY	1.30±0.04	1.12±0.02	3.00±0.01	92.8±7.60	1.73±0.06	5.87±0.14	29.52±0.95
DY	1.20±0.01	1.10±0.03	3.40±0.24	97.3±4.60	2.13±0.20	5.53±0.62	39.03±3.15
BB	0.70±0.01	0.64±0.02	2.00±0.0	17.8±0.82	1.70±0.20	3.23±0.22	52.29±2.99
BY	0.86±0.02	0.78±0.02	2.00±0.01	29.7±0.69	2.47±0.29	4.18±0.35	58.41±2.51
TY	1.36±0.04	1.10±0.01	3.00±0.01	84.8±6.20	2.18±0.15	4.36±0.36	50.84±3.88
BLB	1.54±0.06	1.22±0.03	4.00±0.01	129.7±6.35	2.41±0.33	4.82±0.36	49.06±3.34
BLY	1.36±0.04	1.08±0.03	3.60±0.24	86.4±5.61	1.79±0.30	3.69±0.36	49.30±7.45
CCB	1.68±0.08	1.30±0.03	4.20±0.20	124.4±8.79	2.06±0.20	5.86±0.31	35.96±4.76
CCY	1.76±0.05	1.04±0.05	3.40±0.24	93.6±7.81	1.50±0.09	5.44±0.25	27.65±0.91
AY	1.40±0.06	1.16±0.0	3.20±0.20	103.3±3.95	1.50±0.08	3.78±0.29	39.94±1.32
TPB	1.40±0.05	1.20±0.0	3.80±0.20	113.9±7.25	2.10±0.26	4.32±0.20	47.97±3.97
TPY	1.76±0.08	1.02±0.0	3.40±0.24	102.7±3.24	1.73±0.16	3.50±0.18	49.13±2.75
WY2	1.32±0.03	1.14±0.0	3.00±0.01	97.1±4.64	1.62±0.14	5.44±0.31	29.59±1.27
BKB	1.40±0.03	1.16±0.0	3.80±0.20	101.1±4.24	1.79±0.13	2.73±0.19	65.88±2.99
BKY	1.74±0.10	1.08±0.0	3.60±0.24	117.5±9.11	1.79±0.08	4.53±0.35	39.95±1.56
WB	1.46±0.05	1.22±0.0	4.00±0.01	132.3±7.85	1.93±0.15	3.43±0.16	56.43±3.92
GFB	1.44±0.02	1.22±0.03	3.40±0.24	99.3±4.86	1.98±0.19	3.67±0.29	54.27±3.8
GFY	1.58±0.04	1.10±0.03	3.60±0.24	95.6±6.04	2.16±0.03	4.87±0.27	45.15±3.01
ADL	1.94±0.09	1.04±0.06	3.40±0.24	295.3±5.94	2.13±0.08	3.76±0.18	57.25±3.17
LSD (5%)	0.18	0.12	0.48	119.26	0.56	0.91	9.39
CV%	9.8	8.10	11.50	ns	22.10	16.30	15.90

NL= nut length, NW= nut width, NRN= number of ridges/ nut, 100sw= hundred seed weight, EY= economic yield, BY= biological yield, HI= harvest index

The economic yield ranged between 2.73 and 1.50, with accession KAY having the highest and AY the least. The table revealed that accession WY1 had a biological yield of 5.87g which was the highest among the accessions and accession BKB the least of 2.73g. Accession KAB and CCY recorded harvest index of 67.74 and 27.65, respectively, which happened to be the highest and lowest. The significant differences among the accessions for the yield and yield related trait are a sign of the presence of high degree of genetic variations. This implies the great potential of the accessions for utilization in future breeding programmes.

Cluster analysis

The hierarchical cluster analysis based on the traits evaluated grouped the accessions into six groups (Figure 2). Table 3 shows the clusters, accessions in each cluster them. Table 4 shows the traits that defined each cluster. Cluster II consisted of the largest number of accessions (10) and were characterised by wide nuts and high number of ridges/nuts. Cluster III which had six accessions were characterized by high germination percentages. Cluster I and IV contained the same number of accessions (3) and were high yielding and had high number of tillers per stand.

Cluster V and VI which contain one accession each had high harvest index, economic yield and long nuts. All the groups contained accessions of diverse geographical origin and colour. Figure 4 shows variation in nut shape, size and colour clearly indicating the diversity among the accessions.

Principal component analysis

Variations among the traits were also assessed using principal components analysis (PCA) for the twenty-four

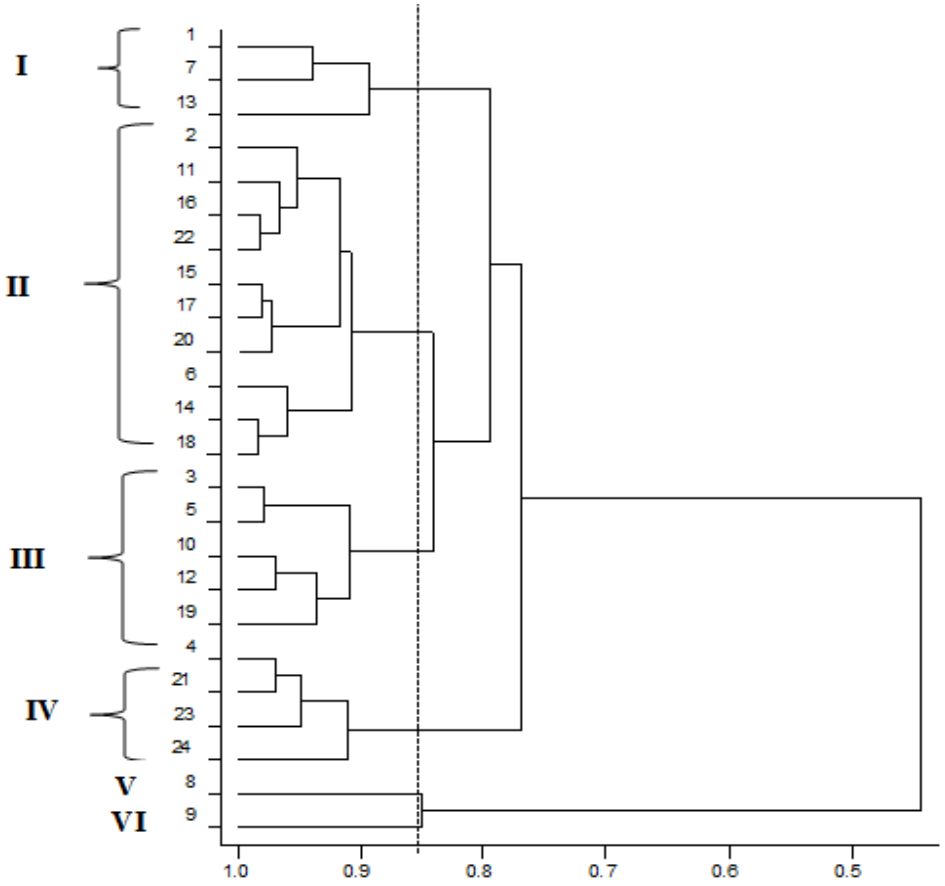

Figure 2. Dendrogram showing the genetic diversity among the twenty-four tigernut accessions in Ghana.

Table 3. Clustering of accessions based on the qualitative traits.

Cluster	Number of accessions	Accessions
1	3	ADS, DY, CCB
2	10	KB, BLB, TPB, GFB, AY, TPY, BKY, WY, CCY, BKB
3	6	KY, KAY, TY, BLY, WY2, KAB
4	3	WB, GFY, ADL
5	1	BY
6	1	BB

accessions. The first five PC accounted for a total of 88.4% variability among the accessions (Tables 5 and 6). PC1 recorded an eigenvalue of 6.8 which explained 45.6% of the entire variation with total number of nuts/stand, number of good nuts/stand and number of detached number of nuts/stand contributing greatly to the variation for this PC. PC2 explained 16.4% of the variation with the eigenvalue of 24.5. PC3, PC4 and PC5 explained 11.1, 8.3 and 7.0%, respectively of the total variation with eigenvalues of 1.66, 1.24 and 1.05, respectively. The biplot which separated the accessions based on PC1 and PC2 shows the accessions scattering in all the quarters and association between traits and accessions (Figure 3). Nuts width, number of ridges/nuts,

Table 4. Means of the agronomic traits for the six clusters of twenty-four tigernut accessionsin Ghana.

Trait	Cluster						Average
	1	2	3	4	5	6	
% Germ	45.50	70.60	76.36	48.00	48.00	50.00	69.13
TS	3.70	3.40	3.09	4.20	3.40	3.00	3.26
AN S	9.80	6.68	4.86	26.00	8.60	2.20	6.58
DNS	11.40	5.44	7.46	22.00	19.20	6.00	8.40
BNS	5.00	3.56	3.66	1.60	3.80	1.80	3.59
GNS	16.20	9.00	9.69	42.00	22.60	7.60	11.88
TNS	20.10	11.84	12.23	43.20	26.20	8.80	14.53
LP	8.30	7.08	7.01	9.00	7.40	7.60	7.26
NL	1.29	1.62	1.45	0.70	0.86	1.94	1.44
NW	1.11	1.23	1.12	0.64	0.78	1.04	1.11
RN	3.20	3.92	3.36	2.00	2.00	3.40	3.35
HSW	94.00	126.58	96.44	17.80	29.70	295.30	104.74
EY	2.02	1.98	1.99	1.71	2.47	2.14	2.01
BY	5.41	4.76	4.34	3.23	4.19	3.76	4.44
HI	37.19	43.06	47.87	52.29	58.41	57.25	46.99

Percentage germination, nut length and hundred nut weights were associated with accessions KB, GFY and ADL were grouped. Attached nuts/stand, good nuts/ stand, total numbers of nuts/stand and detached nuts/ stand were also associated with accessions BB, ADS and DY.

Correlation among the agronomic traits

Pearson correlation was employed among the traits. The highest significant and positive correlation was observed between total number of nuts/ stand and good nuts/stand (r =0.94) (Table 7). Highly significance and positive correlation was also observed between detached nuts/ stand and attached nuts/ stand (r =0.90), total number of nuts/stand and attached nuts/stand (r = 0.89), nut length and total nuts / stand (r = 0.79) and number of ridges/ nut and nut width (r = 0.84). Negative significance correlation was also observed among nut width and detached nuts / stand (r = - 0.88), and harvest index and biological yield (r = - 0.77) (Table 7)

DISCUSSION

The significance differences (<0.05) among the accessions for the agronomic traits is a sign of the presence of high degree of genetic variation. This provides the plant breeder the opportunity to select the best accession for utilization in future breeding programs. Accessions such as BB, ADL, BY and BKB which had high good nut/stand, total nuts/stand, economic yield and harvest index respectively could be included in breeding programmes for varietal development of tigernut. The observed variability could be attributed to the genetic differences among the accessions. Variation in morphological traits among yellow nut sedge and purple nut sedge biotypes has been reported by Tayyar et al. (2003), Bhowmik (1997) and Wills (1998) also reported considerable heterogeneity in morphological among *Cyperus rutundus* populations from around the world.

The clustering of the accessions in the six major groups is an indication of diversity among the accessions of tigernut in Ghana. The grouping of the accessions from same origin and colour into different clusters suggests diversity among accessions within a geographical origin and among accessions beyond geographical origin.

Tayyar et al. (2003), Okoli et al. (1997) and Abad et al. (1998), reported on similar clustering of nutsedge populations on the basis of morphological traits.

The biplot also shows relationship between the accessions and traits evaluated. The observation of the accessions in all the quarters of the biplot suggests a high level of genetic diversity in the accessions evaluated. Concentration should be on the traits that defined PC1 for varietal development of tigernut. Divergence among the purple nutsedge accessions for the morphological traits has been reported by Holt (1994) and Tayyar et al. (2003).

Correlation among traits provides information on the nature and level of association between two pairs of traits

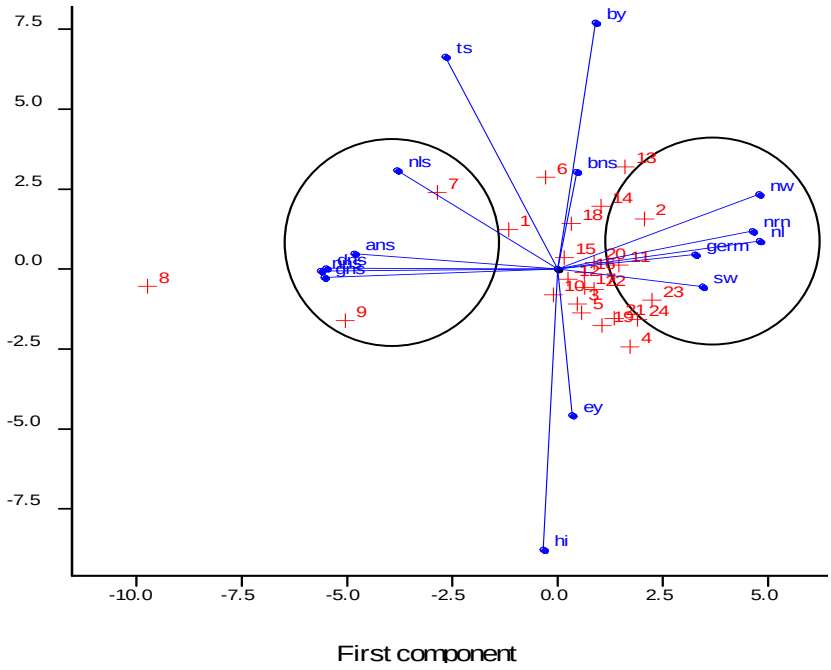

Figure 3. Biplot of the qualitative traits of the twenty-four tigernut accessions in Ghana. NL= nut length, NW= nut width, NRN= number of ridges/ nut, 100sw= hundred seed weight, EY= economic yield, BY= biological yield, HI= harvest index. %Gem= percentage germination, NTS=number of tillers /stand, ANS= number of attached nuts / stand, DNS= number of detached nuts /stand, BNS= number of bad nuts / stand, GNS= number of good nuts /stand, TNS= total number of nuts / stand.

Figure 4. Photograph showing diversity in colour, size and shape of some of the accessions evaluated. A: Asukese Donkorkrom Long; B: Bawku Yellow; C: Krachie Yellow; D: Bowjiase Local Black; E: Bawku Black; F: Twifo Praso Black.

Table 7. Correlation matrix among the agronomic traits for the twenty-four tigernut accessions at 5and 1% probability.

Character	%G	NTS	ANS	DNS	BNS	GNS	TNS	NLP	NL	NW	NRN	100SW	EY	BY	HI
%G	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NTS	-0.19	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANS	-0.41	0.55	1	-	-	-	-	-	-	-	-	-	-	-	-
DNS	-0.53	0.38	0.67***	1	-	-	-	-	-	-	-	-	-	-	-
BNS	0.20	0.22	-0.01	-0.12	1	-	-	-	-	-	-	-	-	-	-
GNS	-0.51	0.49	0.90***	0.09	-0.19	1	-	-	-	-	-	-	-	-	-
TNS	-0.53	0.47	0.89***	0.89***	-0.08	0.94***	1	-	-	-	-	-	-	-	-
NLP	-0.50	0.53	0.60**	0.59**	0.17	0.58	0.64***	1	-	-	-	-	-	-	-
NL	0.32	-0.29	-0.68***	-0.77***	-0.14	0.77***	0.79***	-0.37	1	-	-	-	-	-	-
NW	0.52	-0.14	-0.52	-0.88***	0.29	-0.74***	-0.75***	-0.47	0.56***	1	-	-	-	-	-
NRN	0.41	-0.14	-0.41	-0.87***	0.12	-0.68***	-0.72***	-0.37	0.71***	0.84***	1	-	-	-	-
100SW	-0.02	-0.17	-0.45	-0.58***	-0.21	0.51	0.56**	-0.15	0.73***	0.42	0.53	1	-	-	-
EY	0.03	-0.27	-0.06	-0.07	0.30	-0.10	-0.01	-0.17	-0.09	0.01	-0.04	0.02	1	-	-
BY	-0.06	0.38	-0.19	-0.01	0.33	0.24	-0.15	0.05	0.15	0.26	0.07	-0.02	0.02	1	-
HI	0.05	0.03	0.12	-0.01	0.03	0.08	0.09	-0.14	-0.16	-0.17	0.03	0.05	0.59**	-0.77***	1

=Significant At P<0.005, * = Significant At P<0.001 %G = Percentage Germination, NTS = Number Of Tillers/ Stand, ANS = Attached Nuts / Stand, DNS = Detached Nuts / Stand, BNS Bad Nuts / Stand, GNS = Good Nuts /Stand, TNS = Total Number Of Nuts /Stand, NLP = Number Of Leaves/ Plant, NL = Nut Length, NW = Nut Width, NRN = Number Of Ridges/Nut, 100SW = 100 Seed Weight, EY = Economic Yield, BY = Biological Yield, HI = Harvest.

and it could be possible to improve a trait by the selection of the other pair. The correlation analysis shows significance association among the traits studied which suggest that they can be predicted by using the other. Therefore, traits that showed significance and positive correlation in this study could be improved simultaneously while those that showed negative association will have to be improved independently.

Conclusion

The study was conducted to characterized twenty-four tigernut accessions based on yield and yield related traits. The data shows that there exists a wide range of diversity among the accessions for the traits studied. This should help provide necessary information for the breeding of improved tigernut varieties in Ghana. Promising accessions such as TPY, CCB, BB, DY, ADL, KAY, WY1 AND BKB which recorded high values for the yield and yield related traits should be included in breeding programmes for varietal development of tigernut in Ghana. Also the diverse forms of the accession studied should be conserved at the gene bank in Ghana.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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