KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF AGRICULTURE AND NATURAL RESOURCES
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FLOWERING INDUCTION AND CROSS COMPATIBILITY STUDIES FOR SWEETPOTATO (*Ipomoea batatas*, L.) BREEDING

THE THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME KRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING

BY

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DECLARATION

I hereby declare that, except for the references cited in relation to people's work which have been duly acknowledged in the reference list, this work is the result of my own research and it has not been submitted either in part or whole for any other degree elsewhere.

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We declare that we have supervised the student in undertaking the study submitted herein and confirm that she has our permission to submit.

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Dr J. Sarkodie-Addo
Head of Department

DEDICATION

This thesis is dedicated to my Father, Mr G.B Samba of blessed memory for educating me.

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ABSTRACT

Lack of flowering in some sweetpotato genotypes, and the existence of incompatibilities among them, do present challenges to sweetpotato breeding programmes. To address these in the breeding programmes at CSIR-CRI, flowering induction and cross compatibility studies for sweetpotato breeding were undertaken. Eight sweetpotato varieties; Faara, Sauti, Ogyefo, Apomuden, Hi-Starch, Otoo, Santom Pona, Okumkom and CIP 440390 were used in reciprocal crosses in the first study to determine cross compatibility groups in the Ghanaian crossing block. The varieties Ogyefo,Sauti, Faara,*Ipomoea nil*-cv. Kidachi Asagao and *Ipomoea setosa* Ker. Gawl.were used in the second study of flowering induction, where in Faara, *Ipomoea nil* and *Ipomoea setosa*were used as rootstocks.Ogyefo and Sauti were used as scions in the grafting. The first study was conducted in the field and the second study was conducted in both the screenhouse and field. Data to evaluate the success rate of crosses based on the different cross combinations, on time of day, and month of the year the crosses were made, were obtained from crossing to seed production for all the cross combination groups in the compatibility study and data were obtained from graft establishment to flowering for all graft combinations in the flower induction study. All cross combinations made among the Ghanaian sweetpotato cultivars were compatible.

Assessment of the number of seeds produced per cross suggests that the crosses;Faara x Apomuden, Apomuden x Faara, Faara x Otoo, Otoo x Faara and Santom Pona x Faara were the most compatible among the seed parents. And the best time of day for hybridization was between 6-7a.m. and 7-8a.m. Seed set was highest in August perhaps due to favourable weather condition for hybridization when; the average minimum temperature was 24.0˚C, maximum temperature, 26.7˚C and relative humidity 86.7%.

In the flower induction experiment graft combinations in the field performed far better in flower production thanthose in the screenhouse.

Among the rootstocks, *I.nil* proved to be the most effective in flower induction, followed by*I.setosa*,while Sauti was the best scion plant in the study. Flowering from the different graft combinations started slowly and increased as the time advanced from the first day to the thirty-fourth day of flowering. Afterwards, all the graft combinations in the field were dead and those in the screenhouse stopped flowering, perhaps due to the harsh weather conditions.

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CHAPTER ONE

1.Introduction

Sweetpotato (*Ipomoea batatas* (L) Lam.) is a native of tropical America belonging to the Convolvulaceae family. Sweetpotato is a hexaploid (2n=6x=90) (Austin and Huamán, 1996). Sweetpotato cultivars show wide variation in botanical characteristics and are readily distinguished on the basis of morphological traits.Sweetpotato is a perennial plant which is mostly grown as an annual crop for vines and storage roots in many countries (Andrade*et al*., 2009).

In sub-Sahara Africa, (SSA) sweetpotato ranks the fifth largest staple food crop after rice, wheat, maize and cassava, and the seventh most important food crop in the world (Lin *et al*., 2009).It provides good ground cover, grows on soils with limited fertility and has a short growth period with a high yield potential(Van den Berg and Laurie, 2004). Sweetpotato root is an excellent source of vitamin A, Vitamin C, vitamin B6, riboflavin, copper, pantothenic acid and folic acid(Woolfe, 1992).

Sweetpotato is the only natural hexaploid species known in the genus and its hybridization is largely restricted to crosses within the species (Jones, 1980).Many traits in the species vary over an extensive range, so with proper screening procedures, one can find desirable plant types for almost any need (Martin, 1968).

The section Batatas comprises a polyploid series that includes diploid, tetraploid, and hexaploid taxa that have different sexual compatibility factors and present variable floral morphology and time of flowering (Venkateswarlu, 1980). Sexual compatibility in successful crosses is based on the physiological and cytogenetic traits that are similar between parents andthe definition of the sexual compatibility groups in the section Batatas by some researchers, has already led to several new genotypes in

sweetpotatobreeding programmes (Martin, 1968).Most sweetpotato varieties are self-incompatible and the system of sterility which also expresses in cross-incompatibility can drastically reduce fruit set and seed production in sweetpotato which impedes breeding efforts (Jones, 1967).Each seedling produced during sexual propagation is genetically different from all the others and is potentially a new improved genotype (Ahn *et al*., 2002). The entire plant breeding is based on sexual reproduction, even in asexually propagated species such as sweetpotato, potato and sugarcane, if sexual reproduction occurs, is used to advantage (Singh, 2006).

So far sweetpotato breeding programmes in sub-Sahara Africa based on hybridization exist in only few places such as Uganda (Gibson *et al*., 2008). Most selection of new varieties to date in many places has been based on introduction of existing clones and not products of national hybridization efforts. Where hybridization has been introduced, like Mozambique, new varieties have resulted rapidly, especially with the advent of accelerated breeding scheme (Andrade *et al*., 2009).

Sweetpotato in Ghana is mainly cultivated for the carbohydrate-rich storage roots although the foliage has the potential for use as vegetable and animal feed (Otoo *et al*., 2001). Though the leaves are very rich in minerals and vitamins, the roots are more widely consumed in Ghana where the crop is particularly important in the Central, Volta and Upper East regions.

It is recently that breeding efforts based on hybridization was initiated at the Council for Scientific and Industrial Research-Crop Research Institute (CSIR-CRI) sweetpotato breeding programme and has encountered some challenges with low seed set, the causes of which were not really clear.

The Sweetpotato Action for Security and Health in Africa (SASHA) breeding programme has accelerated sweetpotato breeding in Ghana by initiating sweetpotato population improvement through hybridization. The project also has the challenges of low seed set production from the crossing blocks. These challenges are not just limited to their crossing block but to West Africa in general (personal communication with Dr. Carey.

The main objective of this study was to investigate ways of improving the efficiency of sweetpotato breeding in Ghana by identifying and overcoming some factors that may contribute to low seed production in breeding programs.

The specific objectives were;

a. To determine compatibility among sweetpotato genotypes used in Ghanaian breeding programme
b. To assess seed set in relation to time of day or year that crosses are made
c. To exploit use of grafting to induce flowering in screen house and field environments

CHAPTER TWO

2. Literature Review

2.1 Taxonomy and origin of sweetpotato

Sweetpotato is a perennial, herbaceous anddicotyledonous species of morning glory. It was originally domesticated at least 5000 years ago in or near north-westernSouth America (Austin, 1988; Yen, 1982).Sweetpotato and its wild relatedspeciesare classified in the family Convolvulaceae, genus Ipomoea, section Eriospermum (formerly Batatas), series Batatas (Austin and Huamán, 1996). Linnaeus (1753) described the cultivated sweetpotato as *Convolvulus batatas*. In 1791, the botanist Lamarck described it as *Ipomoea batatas*. It is a hexaploid plant with 2n=6x=90 chromosomes.

Abundant evidence shows that sweetpotato was spread widely through the migration routes of people in the New World tropics before the European discovery of America (Austin, 1988). Based on the analysis of key morphological characters of sweetpotato and the wild *Ipomoea* species, Austin (1988) postulated that sweetpotato originated in the region between Yucatan Peninsula of Mexico and the Orinoco River in Venezuela.

Using molecular markers, the highest diversity of sweetpotato was found in Central America,supporting the hypothesis that Central America is the primary center of diversity and most likely the center of origin of sweetpotato (Huang and Sun, 2000; Zhang *etal*. 2000). Columbus in 1492 brought it to Europe and Portuguese explorers of the sixteenth century took it to Africa, India, Southeast Asia, and the East Indies. Spanish ships brought sweetpotato from Mexico to Philippines in the 16th century. Introduction of the sweetpotato to the Pacific islands apparently occurred in prehistoric times (Yen, 1982).

Fossil carbonized storage root of sweetpotato found in northern New Zealand have been dated back some 1000years (Yen, 1991), which strongly supports the theory of prehistoric transfer, probably by Peruvian or Polynesian voyagers.

The linguistic links between the Quechua and Polynesian names for sweetpotato support the Peruvian origin and human transfer of the Polynesian sweetpotato (Yen, 1982). However, studies based on molecular markers showed that Peruvian sweetpotatoes are not closely related to those from Papua New Guinea (Zhang *et al*., 1998) and are also different from those of Mesoamerica (Zhang *et al*., 2000). It was suggested that the Oceania sweetpotato probably came from Central America, through non-human dispersal (Rossel *et al*., 2001).

2.2 Economic importance and distribution of sweetpotato

Globally sweetpotato is the seventh most important food crop in terms of production (Loebenstein, 2009). It is grown on about 8.2 million hectares producing about 102 million tons with 12.1 tons/ha as an average yields(FAOSTAT, 2010).

Sweetpotato is a valuable food security crop for its producers, because it can enables them to combat shortages and provide coverage in the event of natural disaster(Woolfe, 1992). According to the FAO, 115 countries produced 106,569,572 tons of sweetpotatoes in 2010 butsweetpotatoproduction remains very concentrated, 82.3% of global production being in Asia.China produces about 80% of global production but Africa is coming up (Low et al., 2009).Uganda is one of the most important Africansweetpotato producing countrieswith a harvest that increased from around 2 million in 1999 to 2.83 Mt in 2010. Nigeria (2.83 Mt) and Tanzania (1.4 Mt) with which Uganda, represent half the African supply. This expansion in production in Africa is explained by an increased demand linked to a high human population growth (FAOSTAT, 2012).

Minor players in the global sweetpotato arena are Latin America, which produced 1.97 Mt in 2010 little more than 2% of global supply; Brazil holds first place on the continent, followed by Cuba and Argentina; United States produces relatively little but is the leading global exporter; in Europe only Spain, Portugal and Italy produce sweetpotato, but in very limited quantities on a global scale (FAOSTAT, 2012).

Sweetpotato is one of the most widely grown root crops in Sub-Saharan Africa (SSA) (Low *et al*., 2009). Sweetpotato in developing countries ranked fifth in economic value, sixth in dry matter production, seventh in energy production, ninth in protein production and it has tremendous flexibility of utilization as food and industrial products (Loebenstein, 2009).The greens of Sweetpotatoes are edible and provide an important source of food in Africa (Sierra Leone, Guinea, and Liberia) as well as east Asia (Thottappily, 2009). Over 80% of the sweetpotato produced in SSA is consumed fresh by human beings. The remaining is used for animal feed (Scott, 1991).

Sweetpotatoes role as an important health food is recognised due to high nutrient content with its anti-carcinogenic and cardiovascular diseases-preventing properties (Yoshinaga *et al*., 1999). Awareness of sweetpotato as a healthy food crop is increasing, especially the orange-fleshed sweetpotato which is especially rich in provitamin A carotenoids (Loebenstein, 2009). All varieties of Sweetpotato are good sources of Vitamin C, B2 (Riboflavin), B6 and E as well as dietary fibre, potassium, copper, manganese and iron, and are low in cholesterol (Loebenstein, 2009).Regardless ofthe name ‘sweet’ it is a good food for diabetics as it helps to stabilize blood sugar levels and to lower insulin resistance (Loebenstein, 2009).

In Ghana,it was estimated that a total of 9,622 hectares of sweetpotato was cultivated during the 2012 cropping season. The Upper East region cultivated 5,550 hectares representing 57.7 percent of total area cropped while Upper West and Eastern regions

cultivated 1,157 hectares (12.0 %) and 1,030 hectares (10.7 %) respectively. An estimated total output of 131,990 metric tons of sweetpotatoes was produced. Out of the total production, Upper East region produced the highest of 46,000 metric tons presenting 34.9 percent; Eastern, 34,910 Mt (26.4 %), Upper West, 19,530 Mt (14.8) and Volta region, 15,340 Mt (11.6 %) (MOFA report, 2012). Cassava, yam and cocoyam have been the principal root and tuber crops over the years in Ghana. Sweetpotato was neglected by research in the past, but with the inception of the National Root and Tuber Crops Improvement Project (NRTCIP) in 1988, research attention has been devoted to increasing the production of sweetpotato as a source of dietary energy, vitamins, minerals, proteins and its use as animal feed (FAO and IFAD, 2005). Although it is cultivated mainly for the carbohydrate-rich tubers, the foliage has the potential for use as vegetable and animal feed (Otoo *et al.*, 2001).

2.3 Sweetpotato breeding in West Africa

Sweetpotato as was reported by Yen (1991) was among the 60 common crops that freed slaves returning to Gulf of Guinea brought from Latin America. Its spread was slowly by farmer-farmer diffusion involving no colonial crop dispersal agencies (Loebenstein, 2009). West Africa is traditionally a production zone of root crops because of more or less stable rainy seasons; however, the main root crop is cassava, followed by yam, then sweetpotato (Andrade *et al*., 2009). The‘sweetness’ of sweetpotato makes it less preferred to bland starchy staples like yam, cassava, rice, cocoyam (Loebenstein, 2009).The early breeding of sweetpotato at International Institute of Tropical Agriculture (IITA) (1970-1988) screened out orange-fleshed and sweet type andnew trends seek varied populations to satisfy different end-uses (Akoroda, 2009).

It was reported by Hahn and Leuschner, 1981 thatsweetpotato virus disease (SPVD) pressure is moderate, but with theextension of the dry season, the problem of

weevildamage is on anincrease in West Africa. Breeding is a critical factor of increasing sweetpotato yields and in opening new options fordiversified use of sweetpotato in West Africa. Several SSA countries havesweetpotato breeding programs (Grüneberg*et al.*, 2009). But there is only one National Agricultural Research System (NARS) breeding program with significant medium to long-term population improvement capacity (Andrade *et al*., 2009). NARS partners in the region usually have no or only very small sweetpotato breeding programs,concentrated on adaptive testing of introduced varieties or evaluation of local landraceperformance. With an exception of the Council for Scientific and Industrial Research (CSIR) - Crop Research Institute (CRI) at Kumasi in Ghana whichcapacity for sweetpotatobreeding in Kumasi is similar to that found inRubona, Rwanda. CRI has strong biotechnology and in-vitro laboratory facilities (Andrade *et al*., 2009). However, until fairly recently CSIR-CRI has done little in the way of hybridization.

There are so many challenges associated withsweetpotato breeding in most, if not all of the West African countries such as, breeding sweetpotatovarieties with attributes for dual uses, breeding sweetpotatovarieties for drought-prone and tolerance to weevil damage,breeding sweetpotato varieties for low or no sugar contents and high dry matter content (non-sweet sweetpotato) adapted to West African regions,and the development of methodologies to induce sweetpotato flowering and seed set for breeding purposes and germplasm conservation. Some other challenges are lack of funds for sweetpotato research and development;poor breeding infrastructures,limited facilities for *in vitro* germplasm conservation and insufficient number of technical staff dedicated to sweetpotato work; Lack of government support and scholarships for promising students interested in sweetpotato research(Andrade *etal*., 2009).

2.4Environmentalconditions for flowering and seed set in sweetpotato

Sweetpotato*I.batatas*(Lam.) grows at latitudes ranging from 40˚N and 32˚S. On the equator it is grown at latitudes from sea level to 3000 masl (Huamán, 1987). Light intensity and duration are important for crop growth and development (Rossel *et al*., 2008).Sweetpotato is a short-day plant andflower formation is generally relatedto latitude. Sweetpotato flowers frequently in the tropics while little or no flowering occurs intemperate regions at higher latitudes and even in the tropics, the intensityof flowering varies widely with seasons from poor to abundant, and somevarieties cannotproduce flowers at all (Rheenen, 1965; Eguchi and Gonzalez, 1989).

It is reported by Lam *et al.*, (1959); Campbell *et al.*(1963); Eguchi and Gonzalez, (1989) that flowering can be induced or magnified by using photoperiods of 8-11.5 hours of intense light. And it was also postulated by Reese and Erwin (1997) that some morning glory species thatare very sensitive to short-day can be induced to produce flower buds after exposure to a single 16hours dark period but however, even under long-day conditions, flowering can be stimulated at 24–30 °C. Rossel *et al*. (2008) pointed out thatflowering and fruit set are highest with the temperature of 20-25^{o}C and a relative humidity above 75%. Because of this condition, he said pollinations are more successful in the early morning.There are genetic differences in flowering incidence, as well as strong environmental influences (Grüenberg *etal*., 2005). It is reported by Martin and Jones (1971) that, the seasonal flowering response of cultivars observed in temperate climate may be lost in a tropical climate.The non-flowering habit in somesweetpotato genotypesand breeding lines may be due to chance associations in the original plants used and history of forcing normally non-flowering plants into flower, thus perpetuating the non-flowering plants trait in subsequent generations (Martin and

Jones, 1971).The optimal environmental condition that will hold for all the plantsis difficult to specify because of its variability(Jones, 1980).

2.5.Floral biology of sweetpotato

Sweetpotato flowers are bisexual and within the species, a wide variation is generally observed in theflowering habits of different genotypes (Reynoso *et al.*, 1999). Under normal sowing conditions, some genotypes do not flower, or have scanty flowering and others flower profusely (Rossel *et al.*, 2008).Flowering in sweetpotato is extremely desirable in order to obtain sexual seed from the genotypes for long term gene conservation and breeding purposes (Eguchi and Gonzalez, 1989). Many flower characteristics can help in the verification of cross compatibility among genotypes.The degree of stigma exertion over the stamens for instance, which should be equal in all genotypes of the same compatibility group (Shrenven, 1954; Martin, 1968; Diaz et al., 1995).

Sweetpotato flowers are similar to those of other morning glories with differences occurring in size, shape, and colour. The flowers occur in axillary inflorescences of 1 to 22 buds which open singularly or in groups of two or more (Hsia and Ching-Kuan 1956; Jones, 1980).The buds developed in first, second,and third order but however, single flowers are alsoformed. The flower buds are joined to the peduncle through a veryshort stalk called the pedicel. The colour of the flower varies from white through degrees of lavender of various patterns to complete lavender (Jones, 1980).

Thesweetpotatocorolla is funnel shaped mostlywith white limbs and lavender to light purple throats. The depth of the tube varies from 28 to 63mm and the width of the limb from 26 to 56mm (Jones, 1980). The calyx consists of 5 sepals, 2 outer and 3 inner, that stay attachedto the floral axle after the petals dry up and fall.The androecium consists of five stamens with filaments that arecovered with glandular hairs and that are partly

fused to the corolla(Huamán,1999).The filament length is variable in relation to the position of thestigma and the anthers are whitish, yellow or pink, with a longitudinaldehiscence. The pollen grains are spherical with the surface coveredwith very small glandular hairs. Thegynoecium consists of a pistil with a superior ovary, two carpels,and two locules that contain one or two ovules (Huamán, 1999). The style is relativelyshort and ends in a broad stigma that is divided into two lobes that arecovered with glandular hairs. At the base of the ovary there are basalyellow glands that contain insect-attracting nectar. The stigma is receptive early in the morning and the pollination is mainly by bees. Sweetpotato flowers open soon after daybreak and generally are fading by noon depending on the environmental conditions(Jones 1980).

2.5.1Fruits set and seed yield

The fruit of sweetpotato isthe capsule which is more or less spherical with a terminal tip, and can be pubescent or glabrous. The capsule turns brown when mature and each capsule contains from one to four seeds that are slightly flattened on one side and convex on the other.Sweetpotato seed shape can be rounded, slightly angular or irregular and its colour ranges from brown to black which sizes are approximately 3mm. The embryo and endosperm are protected by a thick, very hard and impermeable testa. Seed germination is difficult and therefore requires scarification by either mechanical abrasion or chemical treatment. Sweetpotato seeds do not have a dormancy period and can maintain their viability for many years (Huamán, 1999).

The best sweetpotato seed yield could beobtained during a period of minimum daily temperature from 12.8 to 18.6˚C, maximum daily temperatures of 23 to 34 ˚C, relative humidity from 62 to 75%, and a photoperiod of about 14.5hours and the high seed set is generally related to good flowering (Jones 1980). Seed set in sweetpotato is reduced when vine growth is rapid and vigorous, therefore low nitrogen fertilizer and low soil

moisture probably improve seed set. Many Sweetpotatoes rarely flower under normal certain environmental conditions, special efforts are necessary if they are to be used in breeding programmes (Martin, 1968). Sweetpotatoes that respond to short-day lengths can be crossed in the greenhouse during cold months and the crossing is generally done during the time when days are about 12hours long and temperatures are not excessively high, about 24 to 30˚C be optimum and night temperatures of 16 to 17˚C to be optimum (Jones, 1980).

2.5.1.1Seed development, harvest and storage

The production of sexual seed in sweetpotato is limited by genetic and environmental factors. The most important genetic factors are duration and intensity of the reproductive period, spore incompatibility, partial or total fertility or sterility, and the structure of the ovary (2 carpels and 2 locules), (Martin, 1968 and Srinivasan, 1977).The hexaploid nature of this species (2n=6x=90) significantly influences the last three factors, while its vegetative propagation favours the preservation of mutations (Ahn *et al*., 2002). The most important environmental factors are the duration and light intensity, temperature, water supply, nutritional imbalance, and pest and disease attacks (Grüenberg *et al*., 2005). Thesweetpotato seeds mature in about one month, a little sooner under hot temperatures and later in cool temperatures. Its seeds are mature when capsules are completely dry and brown. Capsules contain a maximum of four seeds, but the average is much less, about 1.1 to 1.7 reported by (Wang and Burnham, 1968). Mature seeds are about half their maximum green size. The seeds have diameters of 3 to 5mm and are flat on two sides and round on the other (Martin and Cabanillas, 1968). Sweetpotato seeds are usually dark brown or black, but some are tan and others speckled (Jones, 1980).Since the flowering of sweetpotato is over an extended time, seed harvest also extended. Some plants hold mature seeds well and do not need frequent harvesting while others, if not harvested promptly, drop the capsules to the

ground where they rot and allow the seed to sprout (Rossel*et al*., 2008). Seed are threshed by hand and stored in envelopes properly identified with their origin (Jones, 1980).Sweetpotato seeds are very hard and can retain viability for a long period of time. They maintain viability for 20 years or more if they are under a favourable conditionwith relative humidity of10% and temperature of 18˚C (Rossel *et al*., 2008). Germination is very irregular in sweetpotato unless some means of seed scarification is used because of the hard seed coat (Rossel *et al.*, 2008). Percent germination can be increased by putting seeds in water and discarding those that float, a high proportion of those that sink are viable (Jones, 1980).A seed weevil, *Megacerus impiger*Horn. can infest seed under field conditions and during storage. Andfungi such as *Fusarium moniliforme*, among others, and insects such as *Prodiplosis* sp., *Melanogromiza caerulea*, *Nezara viridula*, can attack immature sweetpotato anthers or seeds, in plants growing in the field (Loebenstein, 2009). Satisfactory control for the storage pests has been obtained by enclosing a small segment of household pest strip (20%, 2, 2-Dichloroxinyl dimethyl phosphate) in a plastic bag with the seed for about one week and the control with systemic pesticides in the field increases flowering and seed set (Rossel *et al*., 2008).

2.6Grafting

Several techniques have been developed to promote not only sweetpotato flowering but also fruit and seed production. These include the use of short photoperiod, moderate temperature, limited water supply, grafting, trellises, growth regulators, overwintering, vine girdling, pesticide sprays, soil fertilization, the "bouquet" method, and genetic selection (Rossel *et al*., 2008). In practice, a combination of methods (double method) is typically used, and commonly complemented with the determination of sexual fertility and compatibility of the genotypes to be included in seed production (Rossel *et a*l., 2008). With the ability to induce sweetpotato to flower, breeding programmes switched

from the selection of spontaneous mutant types to selection of new seedlings resulting from open and cross pollinations and with these methods to produce true seed, it is found that factors limiting seed production are reduced (Martin, 1965; Ahn *et al.*, 2002).Some sweetpotato genotypes with desirable characteristics produce few flowers and fruit sets are usually very low.It is recognized that low humidity, the use of certain chemicals, use of certain *Ipomoea* rootstocks, or proper nutrition, and short photoperiod promote sweetpotatoto flower (Kobayashi and Nakanishi, 1982).

Grafting method is one of the most effective techniques of inducing the sweetpotato to flower and seed-set especially under greenhouse conditions when time will be limited to six months or even less (Hsia, 1956; Jones, 1980). Induction of sweetpotato to flower artificially is necessary for breeding purposes and long term gene conservation especially in temperate regions of the world (Martin, 1968). Ahn *et al.*,(2002) reported that flowering of sweetpotato can be artificially induced by grafting and short-day treatment.

Grafting is the art of joining two different plant parts together in such way that they unite and continue their growth as a single plant. The stock is the lower portion of the graft union, while thescion is the upper portion and the place at which both unites is termed as scion or graft union (Bryant, 2006). The ability of two different plants when grafted together to produce a successful union and also to develop satisfactorily into one composite plant is termed as compatibility and the inability of two different plants to do so when grafted together is graft incompatibility (Hartmann *et al.,* 2011).For sweetpotatografting method with short-day treatments, which is the double method was adopted and have contributed considerably to hybrid seeds production (Jones, 1980). Sweetpotato scions grafted onto rootstocks with profuse flowering, blossom early, and in a profuse manner. The most efficient rootstocks are *Ipomoea carnea* subsp. *Fistulosa* Jacq. (Mart. ex Choisy), *Ipomoea nil* cv. "Kidachi Asagao", var. *integriuscula* or var.

limbata in warm climates (Eguchi and Gonzalez, 1989). Some sweetpotato clones may also be used as rootstocks, but with variable results. In all cases, a 'cleft' type of graft is used with a 2- 5 cm sweetpotato scion. A short-day treatment could be initiated approximately after 3 weeks when the tips of the scions are pruned. Best results are obtained when rootstock plants are young and the scions come from mature sweetpotato plants (Lardizabal andThompson, 1988).

2.7Hybridization

Hybridization in plants seeks to ensure pairing of homologous chromosomes and the cross-over events during mitosis and meiosis which can lead to the exchange of segments and graduate into recombination that result in new recombinants (Sharma, 1994).

Equipment needed for controlledhybridizationare; a pair of tweezers,paperclips, small marking tags (about 1.3 to 1.9cm), and some means of protecting the emasculated flowers such as small glassine bags orsoda straws (Jones, 1980). Flower buds that will open the next day are easily recognized because they are much larger than the other buds.

Usually emasculations are done in the afternoon by slitting the corolla into two parts from the sepals through tip with tweezers or any other sharpe object, pulling each half of the corolla down and out of the forefinger and thumb (Rossel *et al*., 2008).The attached stamens will be removed with the corolla and the pistil exposed. The pistil should be protected with a short soda straw pinched over at one end or glassine bag to prevent insect entry.When emasculations are made, buds to be used in self-pollinations and as pollen sources are prevented to be opening the next morning by placing a paper clip or a piece of a soda straw over their tip.

The clips or tips can be knocked off easily by the movement of people, wind or even bumblebees, because of this reason breeders are toprepare more than they expect to use (Rossel *et al*., 2008).

Pollination can be made the next morning as soon as anthers begin dehiscing at about 5:00 to 9:00am (Jones, 1980). The anthers are removed from the protected flowers with tweezers and pollen applied to stigmas of desired female parents. The same flowers used as a male can be selfed by removing the corolla and protecting the corolla against out crossing. If the stigmas and anthers are the same height, self-pollination may be accomplished by shaking the corolla and leaving the straw or paper clip in place. The straw or bag is replaced over the pistil after pollination and the tag attached giving the male parent designation and any other information desired.

Tags of different colours may be used to designate different parental lines or types of crosses. The protective covering should be removed after two days. Crossed buds should be observed closely to prevent younger buds from growing through the string of the tag, which could result in misidentification of cross.Much of the intensive labour required by controlled crossing procedures can be avoided through the use of open-pollination by naturally occurring insects (Rossel *et al*., 2008).

2.8Fertility, sterility and compatibility in sweetpotato

Sexual reproduction is very important because, it is the fastest means to create new genetic variability in different genotypes. It allows the recombination of desirable traits present in different genotypes, such as high productivity, resistance to pests, diseases, and environmental stresses, and industrial or culinary quality, which are present in sweetpotato genotypes (Reynoso *et al*., 1999). The large number of genotypes that is produced by the recombination of these genes is an essential step in creating variation through hybridization (Martin, 1968).

Crossability among sweetpotato genotypes rangesfrom 0 to 100%, but because of incompatibility and limited fertility, lower values are more frequent and, therefore, constitute a serious barrier for genetic improvement and the conservation of the genetic resources of this crop (Martin, 1968 andAhn *et al*., 2002).

The inheritance patterns of this species are better described in quantitative genetic terms than in qualitative terms because of the continuous range of plant types that occur in any lot of seedlings (Jones, 1987).

Several studies of hybridization and crossability at the level of taxonomic sections of sweetpotato have been carried out with relative ease by different researchers concentrating on physiological and cytological barriers between the species (Diaz *et al*., 1995). The quantitative mode of inheritance and hexaploid nature of sweetpotatoinfluences breeding strategies.

Most varieties of this crop are self-incompatible, and because of the obligate out crossing nature of the crop, it has high levels ofheterozygosity (Diaz *et al*., 1995).In spite of the fact thatearly efforts were made to feature the failure of self and cross-pollinations to breed the sweetpotato, not until when it was indicated by Stout (1926) that self-incompatibility might be present and that under certain conditions, other factors may also play their ownroles(Jones, 1967).

It was postulated by Martin (1968) that, the reaction of some morphological features, (stigma, style, ovule), the number of nuclei in the mature pollen grain and the type of cytokinesis during meiosis are all correlated with genetic and physiological aspects of incompatibility. Martin (1968) also postulated that cytological feature that may be correlated with incompatibilityis the hexaploid nature ofsweetpotato.

It is indicated that the best way to proof that poor fertility plays a limited role in poor fruit set in*Ipomoea*crosses, is that genotypes that fail to set fruit in some crosses, can succeed in other crosses using them as both male and female parents with few

exceptions (Martin and Jones, 1971). Except that there isclear evident of chromosomal abnormalities or poor pollen production in the genotype that fail to cross, the cause of failure is probably self-incompatibility (Fujise*et al*., 1955 and Martin, 1968). There is no simple morphological feature to suggest the type of incompatibility in sweetpotato because typical heterostyly is not found in the Convolvulaceae but stamens of thesweetpotatoflowers are frequently different inlengths (Martin, 1968). The length of the style and stamens is varietal characteristics inherited in quantitative manner andthe presence of self-incompatibility or a particular incompatibility phenotype is not associated with these lengths(Hernandez and Miller, 1964; Diaz *et al*., 1995).

Staining of pollen tubes is another diagnostic technique for compatibility.Sterility and limited fertility in hybrids suggest pollen abortion, pollen germination andpollen tube growth failure, poor germination of seeds, and weakness and inviability of seedlings (Jones, 1968 and Diaz *etal*., 1995). Sterility in plantsindicates developmental unbalances, which is probably due to genic and sometimes minor chromosomal differences among parent species of sweetpotato(Brewbaker, 1957 and Jones, 1986). Martin (1968) reported that sterility insweetpotato hybrids may have been fixed by polyploidy and thus maybe impossible to get rid of. Cross-sterile groups of sweetpotatowere classified by complete diallel crosses among varieties (Ando *et al*., 1963, Hernandez and Miller, 1964, Fujise, 1964, Komaki and Chishiki, 1982, Ahn *et al*., 2002).

Fertility in sweetpotato is frequently from 20-50%, although total sterility is not rare (Burnham, 1967; Wang and Burnham, 1968; Jos and Bai, 1985).Sweetpotato incompatibility is said to be sporophytic, which is the main barrier to seed production (Togari, 1942, Martin, 1965; Martin and Cabanillas, 1968). There might be few with gametophytic incompatibility where in the reaction of a pollen of a genotype is determine by itselfand not by the genotype of the plant on which it is produced.

Incompatibilities, sterility and environmental conditions all affect capsule and seed set percentages(Williams and Cope 1967; Ahn*et al*., 2002). The success rateof sweetpotato crosses normally varies from 1 to 47% for different female parents butaverage success of 35% is considered good. Success from selfing rarely exceeds 3% capsule and seed set (Yunoue and Hirosaki, 1975; Wilson *et al.*, 1989; Ahn *et.al.*, 2002).

Jones (1980) reported that different theories have been used to explain the sterility and incompatibility systems and put certain plants into compatibility groups, but none has held up when applied to plants from different origins.Several techniques have been developed to determine whether a particular cross is likely to be successful and which direction of a cross is more likely to be obtained(Togari and Kawahara, 1942; Vimala, 1989).

Genetic markers are not normally used in sweetpotato breeding as a rule, but however parental characteristics often can be recognized in progeny (Villordonand La Bonte, 1995 and Ahn *et al*.,2002).The classification of compatible and incompatible systemsinsweetpotato by Japanese scientists is very practical as reported by Nakanishi and Kobayashi, (1975) that genotypes within each group are incompatible whereas those from different groups are compatible. The representative genotypes from each group have been identified and are known as 'incompatibility testers' which are normally used in crosses whose compatibility needs to be determined(Nakanishi and Kobayashi, 1975).

Sweetpotato cultivars from Peru, Colombia and Brazil exhibit the highest number of incompatibility groups, followed by those from Mexico, the United States, the Philippines, the South-East Pacific Islands, and the remainder of Asia (Reynoso*et al*., 1999).

2.9Cultivation of sweetpotato

Sweetpotato requires warm days and nights for optimal yields. It is sensitive to low temperatures and grows best in the tropical and warm temperate regions wherever there is sufficient water and sunlight. It grows favourably under well aerated and moderate to slightly acidic sandy to sandy-loam soils but has the ability to tolerate harsh soil and climatic conditions and still give a satisfactory yields (Van den Berg and Laurie, 2004). Sweetpotato is typically propagated asexually using vine cuttings and sexual seeds are only used in breeding programs. Maturity period in Ghana varies from under 3 to 5 months depending on variety (Otoo*et al*., 2000).Sweetpotato can also be propagated vegetatively from the sprouts produced by bedding mother roots (Van den Berg and Laurie, 2004).Sweetpotato is most commonly grown on mounds or ridges, and occasionally on raised beds, or on the flat. Deep cultivation enhances root growth and bulking of the sweetpotato roots. Mounds and ridges promote adequate drainage and ease of harvesting (Low *et al*., 2009).It requires from 500-1250 mm rainfall in West Africa. A high rainfall leads to excessive vine development (Low *et al*., 2009).Heavy, poorly aerated soils prevent satisfactory development of storage roots resulting in poor shapes, low yield and make harvesting difficult. Optimum pH for sweetpotato is 5.8-6.0 and the crop can be cultivated in an elevation as high as 1500m above sea level (Obigbesan, 2009).

2.9.1 Production constraints

According to Low *et al*. (2009), there are five major constraints to improved productivity and incomes from sweetpotato among the smallholder sector in Sub-Saharan Africa which includes; the lack of timely access to clean planting material, lack of improved varieties adapted to local environments, damage due to the sweetpotato weevils particularly in drier production areas, insufficient knowledge and use of better agronomic practices and lack of markets.

2.10Sustainable seed system

Improved seed systems have a proven track record in raising productivity of clonal crops such as sweetpotato through the provision of quality planting material and through the efficient dissemination of improved varieties(Low *et al*., 2009). In Sub-Saharan African countries like Ghana, where there are informal seed systems, the same variety is called different names in different places which make management of these varieties very much complicated. A sustainable seed system has several advantages such as ensuring that growers have ready access to adequate quantities of planting material of the varieties they are interested in and at the time they are ready to plant. Okpul*et al*., (2011) reported that in order to maintain superiority of genotypes and, in some cases, health, there needs to be capacity within seed systems for generation, dissemination and multiplication of new stock, new genotypes and or pathogen-free material.

2.11Released varieties of sweetpotato in Ghana

A variety is defined as a taxonomic unit created and maintained by man, that it should have an individuality which can be reproduced over a number of years, and that it should be distinguishable by inherited morphological or physiological characters from other varieties (Bishaw and van Gastel, 2009). The new variety released must be distinct, uniform and stable in all features, including yield. Variety release procedure is a collective term that refers to the released type, the attached terms and conditions, the protocols and administrative procedures used in releasing a new variety for seed production and distribution (Delouche and Goma'a, 1999).Twelve varieties of sweetpotato have been released by the CSIR-Crops Research Institute and one released by KNUSTas follows:Okumkom, Sauti,Faara and Santom Pona; Hi-Starch, Ogyefo, Otoo and Apomuden ; and Patron, Bohye, Dzila Dadanyuie and Ligri in the years 1998, 2005 and 2012 respectively and Tech Santom.Officially released varieties in Ghana are all selections from exotic introductions(CSIR-CRI, 2011).

CHAPTER THREE

3. Materials and Methods

3.1 Site of experiment

This experiment was conducted at CSIR-Crop Research Institute, Fumesuain the Forest agro- ecological zone (FAEZ)in the Ashanti region of Ghana. The work was done in two studies. Study one was done in the national sweetpotato crossing block with nine entries and twentyreplications, in April 2012, where reciprocal crosses were made among the nine sweetpotato parental clones from July to September 2012. The second study was established in October 2012 at the same location both in screenhouse and field.The planting in the screenhouse was done in pots and the field experiment was done at the sweetpotato crossing block.

3.2 Varieties and source of planting materials

The parental clones used for crosses in the crossing block were, Apomuden, Faara, Hi-Starch, Otoo, Sauti, Santom Pona, Ogyefo, Okumkom, and CIP 440390. The varieties used in the second study of the experiment were, Ogyefo, Sauti, Faara, *Ipomoea nil*-cv. Kidachi Asagao and *Ipomoea setosa* Ker. Gawl. Faara, *Ipomoea nil* and *Ipomoea setosa* were used as rootstocks. Variety Ogyefo and Sauti were used as scions in the grafting.The experimental materials consisted of eight released varieties from the CSIR-CRI sweetpotato breeding programme (1998, 2005) and one other genotype CIP 440390. These released varieties were Faara, Sauti, Ogyefo, Apomuden, Hi-Starch, Otoo, Santom Pona, and Okumkom. Sauti and Ogyefo were pathogen-tested materials and were obtained from the Crop Research Institute Fumesua. The two wild relative species of sweetpotato*Ipomoea nil*-cv. Kidachi Asagao and *Ipomoea setosa* Ker. Gawl. Seeds were obtained from theInternational Potato Center (CIP) Lima, Peru.

3.3Cross compatibility study

3.3.1 Preparation of planting materials, land and planting

Land preparation was done in April, using simple hand tools to slash,clear and ploughing of the land. Pelleted chicken manure was applied and ridges were raised with the height of 14 inch in the crossing block. Sweetpotato vines from the field nursery were cut with the length of 30cm from the tip unto the middle portion of the plant prior to planting. Two vines per stand were planted horizontally in rows with 4 nodes buried deep in the soil and in random. The planting distance was 1mx1m within rows and 2m between rows. Each ridge was having all the nine varieties used in the experiment. The crossing block was consisted of 20 ridges which were as well used as replications. Plants were trellised to encourage flowering and facilitate ease of pollination and seed harvest.

3.3.2 Management practices in the field

Hilling in the crossing block started 5 weeks after planting to aid weed control and reduced damage caused by sweetpotato weevil. The hilling procedure consists of pulling soil from both sides and increasing ridge height and width.

The plants were moderately irrigated twice every week as sweetpotato requires less water than most other vegetables. Vines were trimmed regularly especially when the growth was vigorous to enhance fruit and seed set.

Pesticide application was done every two weeks to prevent the attack of fungi and insects. The dosage of 50 ml Durban and 25 ml of confidor were mixed in 15 litres of water in knapsack and spray.

3.3.3Pollination

The pollination among the nine parental clones(Apomuden, Faara, Sauti, Otoo, Hi-starch, CIP 440390, Ogyefo and Okumkom) started on the day of first anthesis and all the crosses were done by hand.Flowers to be used as males and females that would open

the next day were indentified on the previous afternoon and their corollas held closedby placing pieces of soda straw at the tips of the buds to open the next day. Pollinations were carried out in the next early morning between 6:00a.m. to 10:00a.m. Male flowers were excised and corolla removed. Pollen from the anthers was then rubbed on the stigma of the female flower, after removing the straw holding the corolla closed. One flower from the male parent was used to pollinate at least one or two of female flowers to ensure a sufficient amount of pollen on the stigma. Corollas of pollinated females were tied closed with thread to prevent pollination by insects and subsequently, an identification tag with crossing date, time and parents indicated on it was attached to the pedicel of the pollinated flower.Each day over the 3 months period from July through September, crosses were made in varying frequencies depending on the availability of flowers for the individual cross combinations. The cross combination,date and time of crossing were noted for each cross.

3.3.4Seed evaluation and harvesting

Evaluation of seed formation were done as unfertilized flowers fall 2–3 days after pollination and seed capsules form 30–50 days after pollination, depending on weather condition.

Seed harvesting were done periodically before capsules opened to avoid seed losses, as not allplants produce seeds at the same time, and nor were all crossed at the same time. Harvested seeds were kept in paper bags and well labelled. Numbers of seeds were calculated and all data recorded.

3.4 Flower induction by grafting andscreenhouse experiments

3.4.1 Substrate preparation

Substrate was prepared using the proportion of 2:1:1 (sand, manure, and dark soil respectively), sterilization using steam in a boiler, and pots (Top diameter 25cm, base diameter 15cm and height 21.5cm) were filled five (5) days before transplanting.

3.4.2Rootstock and scion preparation

Two of the rootstocks, *Ipomoea nil and setosa* seeds scarification was done by mechanical abrasion using sand paper technique. The seeds were imbibed in water for few seconds and placed in Petri dishes withmoistened filter paper and kept at room temperature for 48 hours before transplanting. The sowing was done two days after germination in plastic pots (Top diameter 25cm, base diameter 15cm and height 21.5cm) and placed on tables in the screenhouse. Vines were cut at the length of 30cm for the two scions, Ogyefo and Sauti from the screenhouse and nursed in the samepots as above together with the one rootstock, Faara and also placed on benches in the screenhouse.

3.4.3 Grafting

The grafting (‘cleft’ type), was done fifty-five (55) days after transplanting. The rootstock seedlings were about 40 cm high and had 8 to 10 leaves, the stem tips were cut off and the stem was split for scion insertion. The scion was about 20 cm long and was cut into the wedge shape to fit into the cleft of the rootstock. The graft was held in place with grafting clips until the union were established. The grafted plants were held under a favourable condition in the screenhouse. The plants were placed in a shade and at the same time having access to the needed sunlight, air, water supply and well protected from the invasion of insect pests. The scions, Sauti and Ogyefo were grafted to Faara, *I. setosa* and *I. nil*stocks to investigate the potentialfor inducing simultaneous flowering of genotypes.

3.4.4Planting layout, density, and distance

The grafting experiment was conducted as a two way factorial design with scion/stock combination, and environments as the factors and was laid out in the randomized complete blocks with two (2) replications in each environment.

3.4.4.1 Screenhouse experiment

The screenhouse experiment was done in two replications. The seedlings planted in plastic pots (Top diameter 25cm, base diameter 15cm and the height 21.5cm) were randomly placed in lots, on benches in the given distance of 25cm between pots.

3.4.4.2 Field planting

Field planting was done sixty-four (64) daysafter seedlingtransplanting and the establishment of the graft,in the same area used for the national sweetpotato crossing block at CSIR-Crop Research Institute.Land preparation involved slashing ofweeds clearing,ploughing and ridges were raised using simple hand tools. The planting was done in only two rows at randomwith a planting distance of 1m within rows and 2m between rows.Each row was having six graft combinations as follows: Sauti-Faara, Sauti-*I.setosa*, Sauti-*I. nil* and Ogyefo- Faara, Ogyefo-*I. setosa*, Ogyefo-*I.nil*. The number of plants in each row was thirty-six (36) and the total plant populationin both screenhouse and field was 216 plants. The experimental treatments were as follows: Rootstocks/scions combinations and planting environments.

3.4.5 Management practices

Bamboo stakes and string were used to support the plant immediately after grafting and for trellising as the plants grow to enhance the upwards vegetative growth, higher exposure to light, foreasier pollination, and collection of capsules and to prevent the attack of soil-borne diseases and insects. Pesticide application was done every two weeks in both the screenhouse and field to prevent the attack of fungi and insects. The

dosage of 50ml dursban and 25ml of confidor were mixed in 15litres of water in knapsack and spray in both the environments.

Irrigation by the use of sprinklers and watering cans was done on regular bases because of the dry spell within the period of the experiment. Ureafertilization was appliedin the field one month after planting and no fertilizer was appliedin the screenhouse.

3.5 Data collection

The following parameters were determined:

Environmental conditions (temperature, precipitation and relative humidity) were obtained by utilizing data from the met station of the CSIR-CRI

Percent germination and numbers of plants established

3.5.1Hybridization

Pollination date

Number of flowers

Number of pollinated flowers

Number of capsules obtained

Number of seeds obtained

3.5.2Grafting

Graft survival

Number of flowers was collected from all the grafted sweetpotatoes every day throughout the two months periods thus, January and February.

3.6Statistical analysis

The Genstat Discovery version 4 was used for data analysis for both studies.Total number of crosses made and seed produced were calculated hourly from 6 to 10 am each day for the hybridization study. Data were subjected to analysis of variance (ANOVA), Lsd and Sed at (0.05) was used to determine separation of treatment means.

CHAPTER FOUR

4. RESULT

4.1 The weather conditions of the experimental area CSIR-Crop Research Institute during the period of study.

The mean relative humidity (RH) as was recorded during the period of study from April 2012 through May 2013, ranged from 56.6% to 89.5%.

Relative humidity during hybridization from July to September were 89.1%, 86.7% and 86.7% respectively and was appropriate for hybridization success, fruit set and seed yield.During the time of flowering, January and February, the mean relative humidity was 56.6% and 65.8% which were below optimal for flower induction, as the optimum RH for flower induction range from 75% and above.

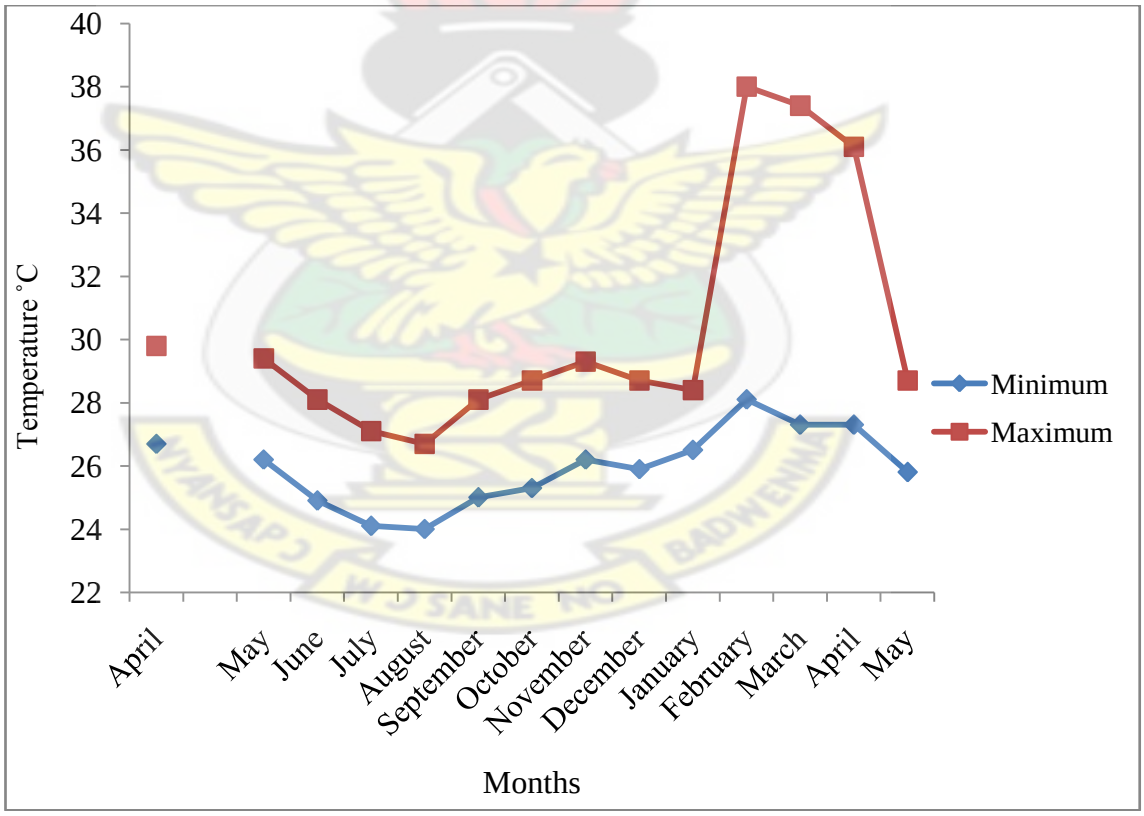

Fig1. Themean daily maximum and minimum temperatureobtained at CSIR-Crop Research Institute from April 2012 through May 2013.

The optimal minimum and maximum temperatures during the period of hybridization, from July through September ranged from 24.0 to 25.0˚C and 26.7 to

28.1˚Crespectively. Minimum and maximum temperature during the period of flowering from January through February 2013 ranged from 26.5 to 28.1˚C and 28.4 to 38.0˚C respectively.The lowest optimum temperature was obtained in August 2012 with the minimum from 24.0˚C and maximum of 26.7˚C and the highest temperature was obtained in February 2013 with the minimum and maximum of 28.1 and 38.0 respectively, which was very high for flowering induction as the minimum daily temperature for flowering induction ranges 12.8 to18.6˚C and maximum from 20.0 to 25.0˚C (Rossel *et al*., 2008).

4.2 Crosses made and seed produced among the Ghanaian sweetpotato clones.

Results of the comparative study of seed setting ability, among the Ghanaian sweetpotatogenotypes ispresented in Table 1. Some of the clones like Hi-Starch and CIP 440390 flowers very late and did not produceseeds. Some other clones, Okumkom and Ogyefo did not flower during the time of crosses,sowere not included in analysis and presentation of results. The genotypes were confirmed to be straight and reciprocally cross-compatible with each other. Highly significant differences occurred among parental cultivars crossed combinationsfor total numbers of seeds obtained and relative percentage mean success.

Table1. Number of crosses made among the Ghanaian sweetpotato genotypes for compatibility study at Kumasi Ghana in 2012

Female x Male	Crosses	Seeds	Ranges of seeds obtained per cross	Relative percentage mean success
Apomuden x Faara	36	22	0-2	60.1
Apomuden x Otoo	6	5	0-3	76.1
Apomuden x Sauti	10	5	0-1	51.1
Faara x Apomuden	46	34	0-3	71.9
Faara x Otoo	25	16	0-2	64.9
Faara x Sauti	11	6	0-1	52.6
Otoo x Apomuden	11	8	0-3	70.7
Otoo x Faara	23	15	0-2	61.3
Otoo x Sauti	8	5	0-2	56.0
Santom Pona x Apomuden	12	8	0-3	67.9
Santom Pona x Faara	22	14	0-2	66.6
Santom Pona x Otoo	7	6	0-3	84.4
Santom Pona x Sauti	11	7	0-2	57.1
Sauti x Apomuden	17	7	0-1	45.3
Sauti x Faara	9	4	0-1	44.4
Sauti x Otoo	9	5	0-2	46.5
Fprobability		**		**
Lsd at 5%		5.9		16.7
CV%		68.2		46.7

Data represent means of monthly totals and relative percentage success for crosses made during hourly intervals between 6 and 10 am daily over the period from July through September

**: highly significant difference at $p<0.01$; Lsd at 5%

Straight and reciprocal crosses were made for the entire cultivars except for Santom Pona which was only used as a female in the crosses because of its non-functional filaments and anthers which produced no pollen grains (male sterile). Although cross-compatibility and seed setting ability among the genotypes showed satisfactory results, the highest relative percentage means of crosses among the cross combinations were obtained from; Santom Pona x Otoo, Faara x Apomuden, Apomuden x Otoo, Otoo x Apomuden and Santom Pona x Apomuden. The highest means of total number of seeds per cross were obtained from; Faara x Apomuden, Apomuden x Faara, Faara x Otoo, Otoo Faara and Santom Pona x Faara respectively. The highest range of seed obtained

per cross are from the crosses made between; Santom Pona x Otoo, Santom Pona x Apomuden, Otoo x Apomuden, Faara x Apomuden and Apomuden x Otoo.

4.2.1Crosses made and seed produced among sweetpotato clones in the Ghanaian crossing block in relation to time of day the crosses were made

The means of total numbers of crosses made, seed produced, and relative percentages over the cross combinations of clones in the crossing block depending on the time of day the crosses were made is presented in Table 2.The analysis of variance(ANOVA), with Lsd at 5% revealed that, highly significant differences occurred among the four periods of times (6-7a.m., 7-8a.m., 8-9a.m. and 9-10a.m.) that crosses were made among the 16 cross combinations.Number of seeds obtained andrelative percentage means,showed a highly significant difference among the individual parental cross combinations of being crossed with regards to the four different times.

Table2. Number of crosses made, among the Ghanaian sweetpotato genotypes during hourly intervals from 6 to 10 a.m.

Times	Crosses	Seeds	Ranges of Seedsobtained per cross	Relative percentage means success (%)
6-7a.m.	21	18	0-3	64.7
7-8a.m.	22	13	0-3	57.3
8-9a.m.	17	8	0-2	47.8
9-10a.m.	6	3	0-1	30.9
Fprobability		**		**
Lsd 5%		2.9		16.7
CV%		62.1		46.7

Data represent means of monthly totalsand relativepercentage success for different cross combinations made over a period from July through September 2012

**: highly significant difference at $p<0.01$; Lsd at 5%

Although the crosses made among the parental cross combination with times were all highly significant, the highest to lowest mean were obtained from the crosses made between 7-8a.m., 6-7a.m., 8-9am and 9-10a.m. respectively. The highest-lowest mean total numbers of seeds obtained with times are in this manner; 6-7a.m., 7-8 a.m., 8-9

a.m. and 9-10 a.m. The highest range of seeds was obtained from the crosses made between 6-7 a.m. and 7-8 a.m. From all indications, there was a decrease in seed success rates as time of the day advanced from 6a.m. to 10a.m.

4.2.2Crosses made and seed produced among sweetpotato clones in the Ghanaian crossing block in relation to the month of year the crosses were made.

Themeans of total numbers of crosses made, seed produced, the range of seeds per and relative percentage of the cross combinations of clones in the crossing block depending on the months the crosses were made are presented in Table 3. Highly significant differences were recognized on number of crosses made, and number of seeds obtained.

Table3. Number of crosses made in relation to the month of year from July throughSeptember among sweetpotato genotypes.

Months	Crosses	Seeds	Ranges of Seed obtained per cross	Relative percentage meanssuccess (%)
July	11	8	0-3	73.0
August	24	15	0-3	62.3
September	15	8	0-2	52.9
Fprobability		**		*
Lsd at 5%		3.3		15.0
CV%		40.2		33.1

Data represent means of totals and relative percentage success for cross combinations

**: highly significant difference at p<0.01; Lsd at 5%; * significant difference at p<0.05; Lsd at 5%.

The highest number of crosses was done in August next to September and July. The mean of the highest number of seeds were obtained in August, next to July and September and the highest relative percentage mean success was obtained in July.The range of seed set per cross was highest in July and August. The numbers of crosses made in each month were determined by the ability of the cultivars to produce flowers. Therefore, the number of crosses may be increasing, thus the probability of getting more seeds is high as well.

4.3 Results for flower induction study

The results of the flowering induction experiment are presented in figures 1 to 3. By considering means separation of the statistical analysis of variance, standard error differences (Sed) at probability of 0.05levels were used. Percentages of graft survival of various graft combinations in different environments (field and screen house) are spell out in the discussion of this study.

Highly significantdifference (P< 0.05) occurredbetween the total number of flowers obtained in the field and screen house. There was a significant difference (P<0.05) between the total number of flowers recorded from the various graft combinations in both field and screen house. The graft combination, *I.nil* /Sauti was significantly increased in the number of flowers than others. Significant difference (P<0.05) occurredamong the total number of flowers obtained per plant, for different rootstocks/scions except for graft combination of *I.nil*/Ogyefo and *I.setosa*/Ogyefo in both the environment.

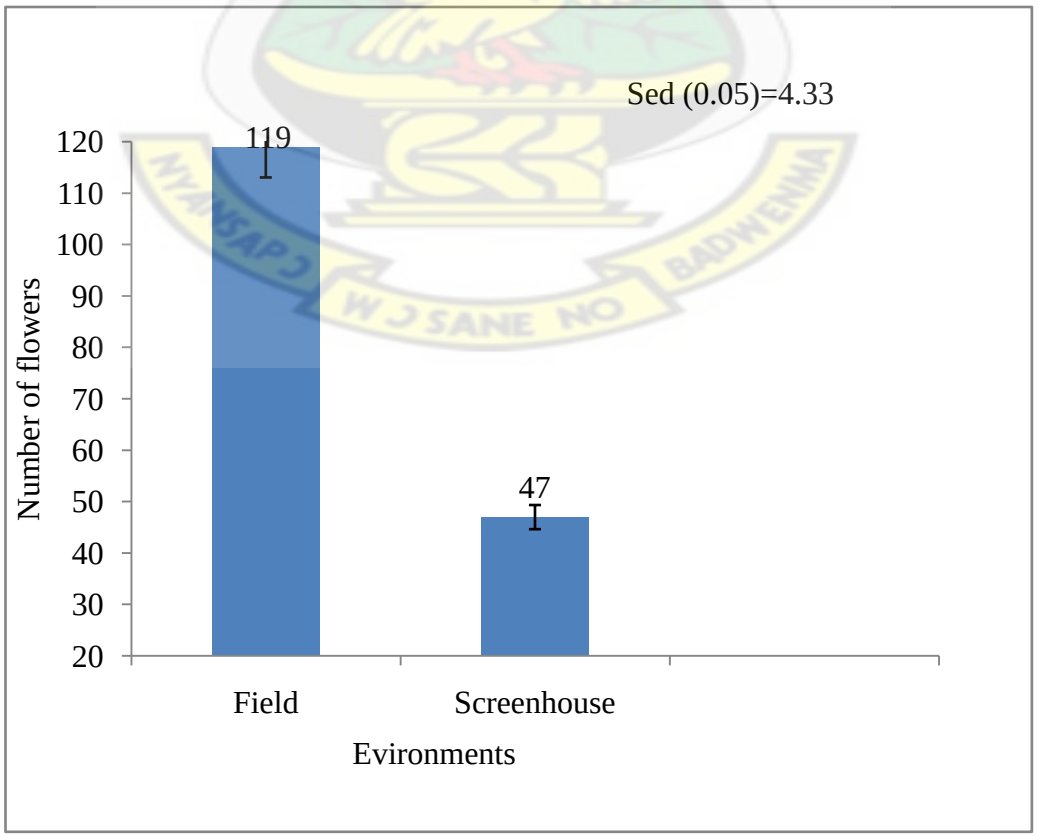

Fig2. The total number of flowers obtained in both the field and the screen house at CRI in Kumasi, Ghana.

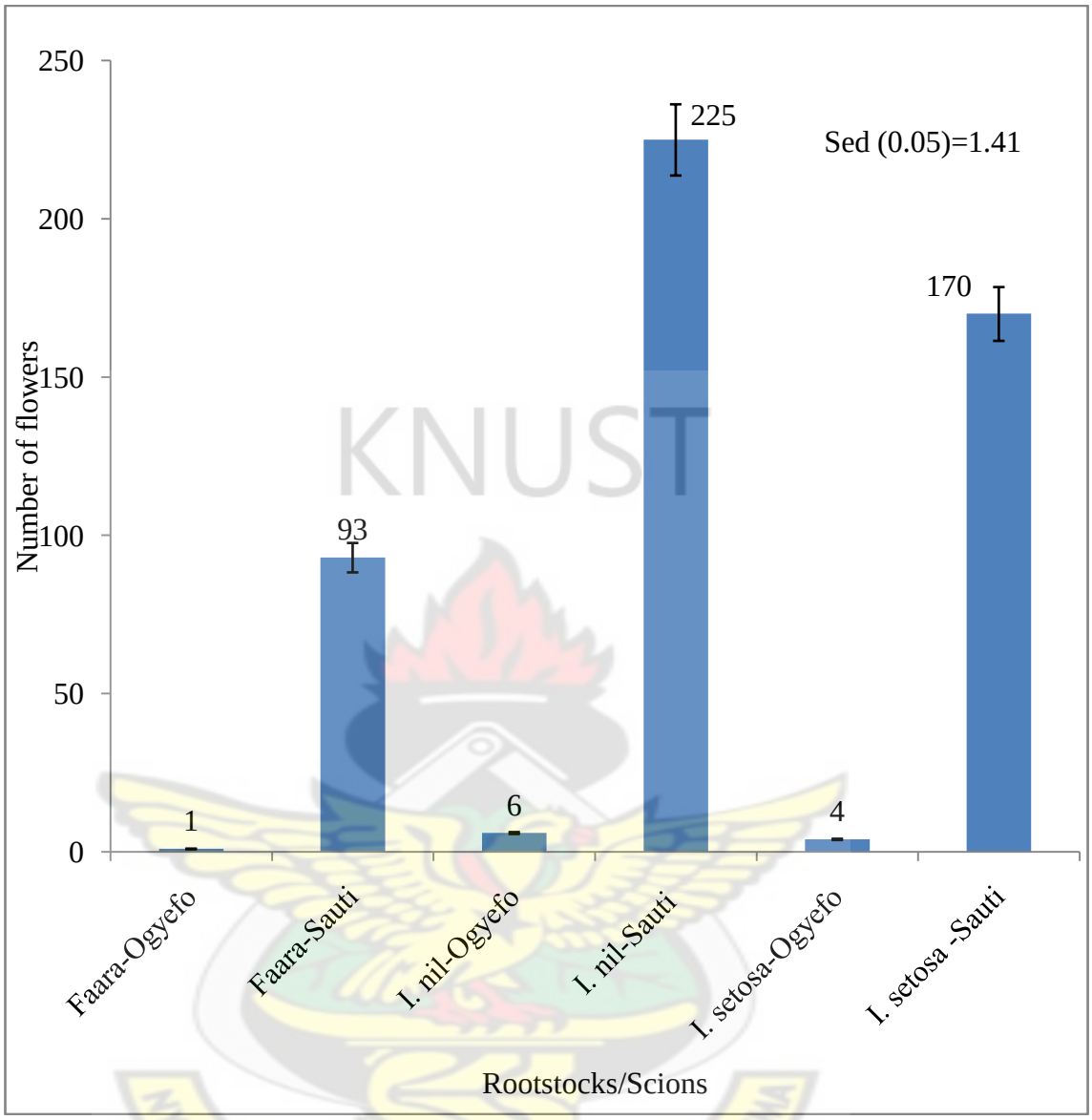

Fig.3Total number of flowers produced from rootstocks/scions combinationsin screenhouse and field.

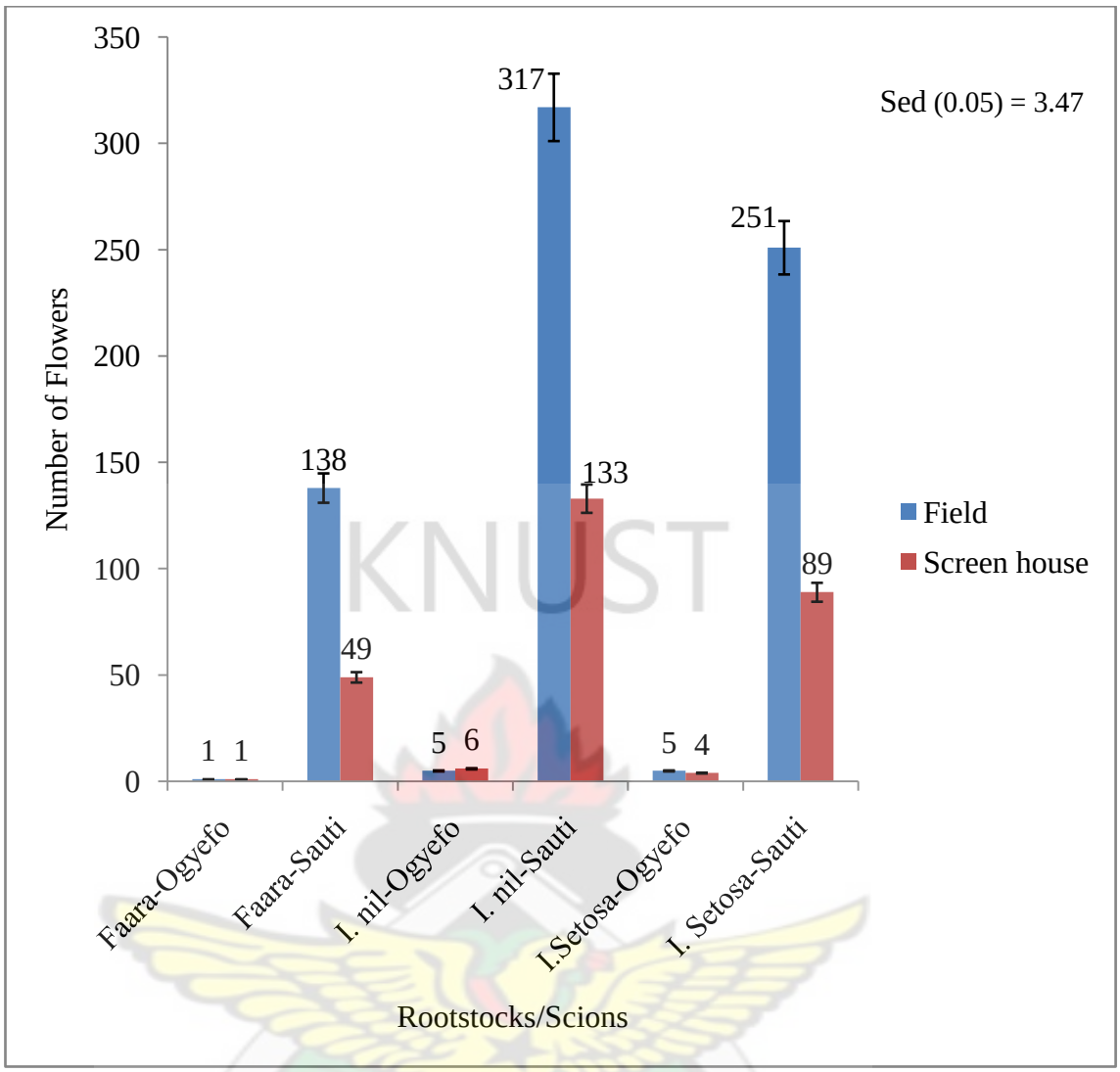

Fig.4 Total numbers of flowers obtained for different Rootstocks/Scions combinations of grafted sweetpotatoes in screenhouse and field.

CHAPTER FIVE

5. DISCUSSION

Very little progress can be made in plant breeding without sexual reproduction.Induction of flowering by grafting is aproven method for overcoming the problems of limited flowering and seed set among parental genotypes of sweetpotato breeding programmes in Ghana (Martin and Jones, 1971; Kobayashi and Nakanishi, 1982).The determination of cross compatibility groups among crop varieties is very important as it enablesthe breeder to transfer desirable recombinant novel traits present in one genotype into the genetic background of other desirable varieties lacking the trait (Martin 1968 and Ahn *et al*., 2002). Flowering in sweetpotato according to Martin and Jones (1971), is extremely desirable in order to obtain sexual seed from the genotypes for long term gene conservation and breeding purposes. But wide variation is generally observed in the flowering habits of different sweetpotato genotypes. Martin and Jones (1971) suggested that, special efforts are to be made by researchers to invent techniques to promote flowering in sweetpotato for the improvement of its breeding.

It was reported by Martin (1968) and Ahn *et al*., (2002) that, crossability among sweetpotato clones ranges from 0 to 100% but because of incompatibility and limited fertility, lower values are more frequent and therefore constitute a serious barrier for genetic improvement of the crop.Martin (1968)and Srinivasan (1977) reported that, the most important genetic factors that limitseed production in sweetpotato are duration and intensity of the reproductive period, spore incompatibility, partial or total fertility or sterility and the structure of ovary. Seed production in sweetpotato is also affected by some environmental factors.Grüneberg*et al.*(2005) highlighted the importance of some of these environmental factors, viz. light intensity and duration, temperature, relative humidity, nutritional imbalances, and pest and diseases attacks.

Earlier reports by some researchers, (Edmond and Ammerman, 1971; Jones, 1980 and Rossel *et al*., 2008), suggested that, the minimum daily temperature for flower induction and seed set in sweetpotato is 12.8 to 18.6˚C and the maximum is 20 to 34˚C and a relative humidity above 75%. In this study, the minimum temperature was 24.0 to 28.1˚C and the maximum was 26.7 to 38.0 ˚C, which was higher than the optimum.

5.1Cross compatibility among Ghanaian sweetpotato cultivars

Incompatibility groups appeared to be absent among Ghanaian sweetpotato cultivars. This shows that sweetpotato breeding could be easily conducted through hybridization procedures without much difficulty, using the parental lines usedin the present study.

Allthe genotypes were observed to be straight and reciprocally cross-compatible with each other as seed set percentages amounted to 44.4-84.4 in the straight and reciprocal crosses among the genotypes. This finding is in contrast to numerous reports of cross incompatibility among sweetpotatogenotypes (Martin, 1968; Jones and Martin, 1967; Yunoue and Hirosaki, 1975; Wilson *et al.,* 1989; Ahn *et al,* 2002), who reported that, the success rate of sweetpotato crosses normally varies from 1 to 47% for different female parents but average success of 35% is considered good.The most compatible groups among the seed parents were as follows; Santom Pona x Otoo, Faara x Apomuden, Otoo x Apomuden, Santom Pona x Apomuden and Santom Pona x Otoo respectively, based on the means of the total number of seeds per cross and relative percentagesobtained among the various cross combinations of cultivars over time.Assessment of compatibility should not be based on numbers of crosses made since that is a reflection of profuseness of flowering, but should be based on success (seeds obtained) of crosses when made.

The varieties Hi-starch and CIP 440390 flowered very late and this was the reason why no seeds were obtained from their crosses and the cultivars, Okumkom and Ogyefo did

not flower at all. Including these varieties in a similar study, the possibility of using grafting method would help to synchronize flowering.

It was also observed that the variety Sauti, when used as a female parent, most of the pollinated flowers abortedone and two days after pollination. Thus in all the crosses where Sauti was made as a female parent,the average number of seed set was below 50 %.It is advisable therefore to use variety Sauti as a male parent in hybridization.

It was also observed that Santom Pona among the cultivars is male sterile and can only be used as female parent in pollination, and it was observed to be highly compatible with the rest of the cultivars, and with a very good attributes of high success rate in crosses and seed setting ability. The value of Santom Pona as a progenitor should be evaluated in progeny tests. The genetics of male sterility of Santom Pona could be investigated for possible broader application in sweetpotato breeding.

5.1.1 Success rate and seed setting ability in relation to time of hybridization and environment

It was observed in this study that, the best time for all the attributes measured wasbetween 6-7a.m., followed by the crosses made between 7-8a.m., 8-9a.m. and 9-10a.m. This indicates that, the crosses made between 6-7a.m. has higher tendency of hybridization success rates and seed setting ability among the Ghanaian sweetpotato cultivars. This observation agrees with the findings of Rossel*et al*. (2008) who reported that fruit and seed set in sweetpotato is highest with minimum daily temperature from 12.8 to 18.6˚C and maximum daily temperature of 20-25˚C and a relative humidity above 75%, because of these conditions, he said pollinations are more successful in the early morning.

The differences in the total number of crosses made for the various times of the study could be attributed to the delay in profuse flowering of some of the varieties. To obtain

more balanced approach to the study in future, date of planting for the varieties should be staggered to obtain synchrony in flowering. The data on number of seeds obtained in the crosses, however, normalized for differences in numbers of crosses made, and allowed differences in seed set to be detected according to the time of day the crosses were made. The results of this study indicates that limited seed production in the CSIR-CRI crossing block might have been due to failure to conduct crosses early enough in the day. Hence controlled crossing should begin as early as possible in the day and not continue beyond 9 a.m.

Apart from the cross compatibilities that showed among the entire cross combinations of the cultivars, more crosses were made between some cross combinations because of the profuse flowering habit in the cultivars, during the pollination period. Theresult on the observation here revealed that the numbers of crosses made in each month were determined by the ability of the cultivars to produce flowers.August was the best month for all investigated attributes. The availability of more flowers in a particular month, could translate to more seeds, as more flowers could be pollinated.The trend in the monthly crosses and seed set successes could be related to vigour and profuseness in flowering of the plants in that particular season followed by slow "tapering off" of flowering as the season progresses. This result is in agreement with the findings of Jones (1980) who reported that, the best seed yield was obtained by Folquer in 1974 during a period of minimum daily temperature from 12.8 to 18.6˚C, maximum daily temperatures of 23 to 34 ˚C, relative humidity from 62 to 75%, and a photoperiod of about 14.5 hours in Argentina, and the high seed set during that period was related to good flowering.It is worth noting that, plants that flowered in August probably were induced to flower earlier. Generally, conditions during the period of this study were apparently favourable for flowering and seed set production. This is indicating that low success rates experienced in 2011 in the CSIR-CRI crossing block were not likely to be

the norm, and that crossing success rate is not likely to be a constraint to sweetpotato breeding at CSIR-CRI, or probably, elsewhere in West Africa.

5.2 Flower induction through grafting

The percentage graft survival for the different scions/rootstocks indicates good graft compatibility among the graft combinations. The different scions and rootstocks produced successful unions and developed satisfactorily into composited plants both in the screen house and field (Hartmann *et al*., 2011). The survival rate was higher in the screen house than the field. This can be partly attributed to harsh and direct sun light on the young seedlings in the field, exposure to pest and diseases and moisture stress,whereas the screenhouse was more confined and protected from direct sun light and the invasion of pests and diseases. However, all of the grafts were made in the screenhouse, and then some of the genotypes were transferred to the field, where some of them died.

The result of observation in relation to the total numbers of flowers obtained in both the environments of study showed that, there were more flowers obtained from the graft combinations that were in the field than in the screen house. The environmental conditions maybe one of the reasons for low flower production in the screen house that is, shades effect (the screen house was covered 50% shade, which in retrospect, should have been removed to perhaps induce greater flowering; light deficiency; high degree of temperature; and low level of relative humidity at the time of the experiment and anti-affinity problems (that causes withering of leaves) in the screen house. It was earlier reported by Kobayashi and Nakanishi, (1982) that all the above mention factors can impede flower in induction.

The intensity of flowering distribution by the different rootstocks/scion combinations per plant in different environments (field and screenhouse)indicates that, plants in the

field did better in flower production than those in the screenhouse.This result is in agreement with Yoshihara *et al*. (2000) who reported that, there are variations in inducing flowering in different sweetpotato varieties and individual plants in different environment. Earlier report by Grüenberg *etal*. (2005) also pointed out that, there are genetic differences in flowering incidence, as well as environmental influences. It was also reported by Martin and Jones (1971) that seasonal flowering responses of cultivars are different with climates and environments.

In considering graft combinationsamong the different rootstocks used, *I.nil* proved to be the best in flower induction in both the environments, next to *I.setosa* and Faara the least.This result is in agreement with the report made by Eguchi and Gonzalez, (1989) that *Ipomoea nil* cv. "Kidachi Asagao is one of the most efficient rootstocks used for flower induction.

Among the scions, Sauti was the best and Ogyefo the least. This is in line with the report from the work done by Martin and Jones, (1971) that the non-flowering habit in some sweetpotato genotypes and breeding lines maybe due to chance associations in the original plant used and history of normally forcing non-flowering plants to flower, thus perpetuating the non-flowering traits in subsequent generations.

A critical control missing from the trial was the evaluation of flowering in non-grafted plants to know if, for example, grafting had any effect at all on the induction of flowering in Ogyefo. Given that Ogyefo was not observed to flower in ungrafted plants in the CSIR-CRI crossing block, it appears that grafting may have induced Ogyefo to flower. However, grafting certainly did not induce profuse flowering, but rather induced only a few flowers. The fertility of the Ogyefo flowers was not evaluated.

In future studies, flowers induced in shy-flowering plants such as Ogyefo, should be used in crosses. However, based on these limited results, it appears that grafting to *I. nil* can be recommended to boost flowering of clones that flowers with an intermediate level of profuseness and to stimulate some flowering in clones in which flowering is very shy. Combining grafting with earlier planting of the late-flowering clones could be another option to maximize chances for using important, but non-flowering varieties in breeding.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

Most selection of new sweetpotato varieties today in sub-Sahara Africa, including Ghana has been based on introduction of existing clones and notproducts of hybridization efforts. Where hybridization has been introduced, new varieties have been resulted rapidly. It is recently breeding efforts based on hybridization was initiated at CSIR-CRI sweetpotato breeding programme and has encountered challenges with low seed set which causes is not clear.This study was relevant to determine the causes of some of these challenges.It can be concluded from the present study that:

1. Incompatibility was absent among the parental clones used in the hybridization study.All the genotypes used were compatible to each other. The most compatible groups among the seed parent were as follows; Faara x Apomuden, Apomuden x Faara, Faara x Otoo, Otoo x Faara and Santom Pona x Faara respectively.
2. In the crosses made to determine cross compatibility, fruit and seed setting ability among the Ghanaian sweetpotato cultivars in relation to time of day the crosses were made showed that, the best time of day for hybridization ranges between 6-9 a.m. and on crosses should be made beyond 9a.m.
3. Not all sweetpotato cultivars are functional as both male and female, Santom Pona among the sweetpotato parental clones was found to be male sterile and can only be used as a female parent in hybridizations.

4. Because of the variations in the time of flowering among sweetpotato genotypes,varieties CIP 440390, and Hi Starch flowers very late. Okumkom and Ogyefo did not flower during the study period. Sauti can flower but later than the other varieties, which has high rate of flower abortion after pollination. Sauti is best used as a male parent rather than a female parent in hybridizations.
5. Flower induction study through grafting showed that thegraft combinations in the field produced more flowers than their counterparts in the screenhouse. Among the rootstock plants, *I.nil* proved to be the most effective in flower induction, next to *I.setosa*and Sauti was the best scion plant in the study.

RECOMMENDATION

It is recommended that furtherwork be carried out to identify both self and cross-compatibility groups, including cytological studies among the Ghanaian sweetpotato cultivars.

It is recommended that, ifCIP 440390 and Okumkom are to be included in breeding programmes, their date of planting will be earlier than the rest of the cultivars and variety Sauti is best used as a female parent in hybridization.

It is recommended that further grafting work be carried out on an appropriate time to induce the almost non-flowering sweetpotato cultivars Okumkom and Ogyefoto profuse flowering for breeding purposes.

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APPENDIX

1. Analysis of variance for flower induction in two Ghanaian sweetpotato cultivars by grafting

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Site	281.24	1	281.24	<0.001
Graft combination	46965.05	5	9393.01	<0.001
Site and graft combination	9173.87	5	1834.77	<0.00
Site x graft combination x Time	3077.79	165	18.65	<0.001

Table of predicted means for Site

Site	Field	Screen
119.48	46.83	

Standard error of differences: 4.332

Table of predicted means for graft combinations

Graft combinations	Fa-Ogy	Fa-Sa	Nil-Ogy	Nil-Sa	Se-Ogy	Se-Sa
	1.12	93.28	5.63	224.7	4.46	169.65

Standard error of differences: 1.408

Table of predicted means for Site and graft combinations

Graft combinations	Fa-Ogy	Fa-Sa	Nil-Ogy	Nil-Sa	Se-Ogy	Se-Sa
Site						
Field	1.46	137.90	5.22	316.66	4.85	250.76
Screen house	0.78	48.66	6.04	132.88	4.06	88.54

Standard errors of differences

Average:	3.468
Maximum:	4.698
Minimum:	1.992

Average variance of differences: 13.84

Table of predicted means for Site x graft combination x Time

Site	Time / Combn	1	2	3	4	5	6
Field	Fa-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Fa-Sa	0.00	0.00	5.50	15.50	25.50	35.50
	Nil-Ogy	0.00	1.00	1.50	1.50	1.50	1.50
	Nil-Sa	5.50	16.00	34.50	69.50	100.00	118.00
	Se-Ogy	0.00	1.00	1.00	1.00	1.00	1.50
	Se-Sa	1.00	6.00	13.00	31.00	50.00	70.50
Screen house	Fa-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Fa-Sa	1.50	4.50	7.50	10.50	15.00	19.00
	Nil-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Nil-Sa	7.00	15.50	22.50	31.50	40.00	48.50
	Se-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Se-Sa	4.00	9.00	14.00	19.00	25.00	32.00

Site	Time / Combn	7	8	9	10	11	12
Field	Fa-Ogy	0.00	0.00	0.00	0.00	0.00	0.50
	Fa-Sa	47.00	57.00	66.50	77.00	85.00	91.50
	Nil-Ogy	1.50	2.00	2.00	3.00	3.00	3.00
	Nil-Sa	161.50	180.50	195.50	225.00	234.00	242.50
	Se-Ogy	1.50	2.00	3.00	3.00	3.00	3.00
	Se-Sa	89.00	116.00	138.50	157.00	177.50	186.00
Screen house	Fa-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Fa-Sa	22.00	25.50	27.50	29.50	31.00	34.00
	Nil-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Nil-Sa	57.00	66.00	72.50	82.00	90.00	100.00
	Se-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Se-Sa	37.50	44.00	48.00	54.00	59.00	66.00

Site	Time / Combn	13	14	15	16	17	18
Field	Fa-Ogy	0.50	1.00	1.00	1.00	1.50	1.50
	Fa-Sa	98.50	103.00	107.50	112.00	120.50	128.50
	Nil-Ogy	3.50	3.50	3.50	4.00	5.00	5.50
	Nil-Sa	254.00	279.50	292.00	309.00	327.50	339.00
	Se-Ogy	3.00	3.50	4.00	5.00	5.00	5.00
	Se-Sa	205.00	218.00	224.00	230.00	236.00	261.00
Screen house	Fa-Ogy	0.00	0.00	0.00	0.00	0.00	0.00
	Fa-Sa	38.50	41.00	43.00	44.50	47.00	47.50
	Nil-Ogy	0.00	1.00	1.50	4.50	4.50	5.00
	Nil-Sa	109.50	116.50	122.50	129.00	134.00	139.00
	Se-Ogy	0.00	0.50	1.50	2.50	3.50	3.50
	Se-Sa	72.50	77.00	80.50	84.50	88.50	90.50

	Time	19	20	21	22	23	24

Site	Combn						
Field	Fa-Ogy	1.50	1.50	2.00	2.50	2.50	2.50
	Fa-Sa	138.00	143.50	148.50	154.00	167.50	188.50
	Nil-Ogy	5.50	5.50	5.50	6.50	7.50	7.50
	Nil-Sa	356.00	364.50	373.00	382.00	408.50	424.50
	Se-Ogy	6.00	6.00	6.00	6.00	6.00	7.00
	Se-Sa	273.50	290.50	297.00	302.00	324.50	342.00
Screen house	Fa-Ogy	0.50	0.50	1.00	1.00	1.00	1.00
	Fa-Sa	50.50	54.00	56.50	60.50	63.50	65.50
	Nil-Ogy	6.50	7.00	9.00	9.50	10.00	10.50
	Nil-Sa	144.50	151.00	157.00	164.00	173.50	180.00
	Se-Ogy	3.50	4.00	6.00	7.50	7.50	8.00
	Se-Sa	95.00	99.50	103.00	108.50	112.50	117.00

	Time	25	26	27	28	29	30
Site	Combn						
Field	Fa-Ogy	2.50	3.00	3.00	3.00	3.00	3.00
	Fa-Sa	205.00	220.50	231.00	237.00	250.50	261.00
	Nil-Ogy	8.00	9.00	9.00	9.00	9.00	9.50
	Nil-Sa	431.00	450.50	465.00	474.00	491.00	522.00
	Se-Ogy	7.00	7.00	7.00	8.00	8.00	8.00
	Se-Sa	357.50	369.50	391.50	409.50	424.00	435.00
Screen house	Fa-Ogy	2.00	2.00	2.00	2.00	2.00	2.00
	Fa-Sa	68.00	70.00	73.00	78.00	80.50	82.00
	Nil-Ogy	11.00	12.00	13.50	14.00	14.00	14.00
	Nil-Sa	185.00	191.00	199.50	209.00	214.50	219.50
	Se-Ogy	8.00	8.50	9.00	9.00	9.00	9.00
	Se-Sa	122.50	128.50	132.00	139.00	143.50	148.50

	Time	31	32	33	34
Site	Combn				
Field	Fa-Ogy	3.00	3.00	3.00	3.50
	Fa-Sa	269.00	289.00	301.50	308.00
	Nil-Ogy	9.50	10.00	10.00	10.00
	Nil-Sa	538.50	559.00	566.00	577.50
	Se-Ogy	9.00	9.00	9.00	9.50
	Se-Sa	450.00	471.50	483.50	495.00
Screen house	Fa-Ogy	2.00	2.00	2.00	3.50
	Fa-Sa	85.00	89.00	92.50	97.00
	Nil-Ogy	14.50	14.50	14.50	14.50
	Nil-Sa	229.00	233.50	240.00	244.00
	Se-Ogy	9.00	9.50	9.50	9.50
	Se-Sa	157.00	162.50	167.00	169.50

Standard errors of differences

Average:	11.99
Maximum:	12.37
Minimum:	11.61

Average variance of differences: 143.9

2. Analysis of variance for crosses made among the Ghanaian sweetpotato cultivars

Variate: Seed per cross

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Month stratum	2	0.57069	0.28535	4.61	
month.*Units* stratum					
Female x Male	15	2.65164	0.17678	2.86	<.001
Time	3	7.50825	2.50275	40.48	<.001
Female x Male x Time	45	7.97260	0.17717	2.87	<.001
Residual	126	7.79073	0.06183		
Total	191	26.49391			

Tables of means

Variate: Seed per cross

Grand mean 0.446

Female x Male	Apomuden x Faara	Apomuden x Otoo	Apomuden x Sauti
	0.453	0.514	0.340
Female x Male	Faara x Apomuden	Faara x Otoo	Faara x Sauti
	0.653	0.671	0.437
Female x Male	Otoo x Apomuden	Otoo x Faara	Otoo x Sauti
	0.457	0.536	0.422
Female x Male	Santom Pona x Apomuden	Santom Pona x Faara	Santom Pona x Otoo
	0.508	0.523	0.404
Female x Male	Santom Pona x Sauti	Sauti x Apomuden	Sauti x Faara
	0.361	0.339	0.215
Female x Male	Sauti x Otoo		
	0.307		

Time	6-7am	7-8am	8-9am	9-10am
	0.694	0.546	0.384	0.161

Month	July	Aug	Sep
	0.436	0.518	0.385

Least significant differences of means (5% level)

Table	Female x Male	TimeFemale x Male x Time
rep.	12	48	3
d.f.	126	126	126
l.s.d.	0.2009	0.1004	0.4018

Stratum standard errors and coefficients of variation

Variate: Seed per cross

Stratum	d.f.	s.e.	cv%
month	2	0.0668	15.0
month.*Units*	126	0.2487	55.7