

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND
TECHNOLOGY,**

KUMASI

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

GENETIC STUDY OF EXTRA-EARLY MATURITY IN COWPEA

(*Vigna unguiculata* (L.)WALP.)

EMMANUEL YAW OWUSU

BSc. (HONS) AGRICULTURE TECHNOLOGY

NOVEMBER, 2015

KNUST



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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF**

MASTER OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING)

NOVEMBER, 2015

DECLARATION

I hereby declare that, this thesis is the result of my own research and has not been submitted either in part or whole for other degree elsewhere and that all references have duly been acknowledged.

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DEDICATION

I dedicate this thesis to my cherished wife Linda Owusu and my beloved sons (Eugene and Eric) for being the inspiring force behind my success.



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ABSTRACT

Three field experiments were conducted to investigate the genetic basis of extra-early maturity in cowpea by incorporating extra-early maturity genes from a land race (Sanzi) into locally adapted improved medium maturity cultivar (Padi-Tuya). Ten progenies derived from the cross were evaluated using RCBD with three replications. The genotypes showed highly significant ($p < 0.01$) variability for days to 50% flowering, days to first flower initiation, days to 90% pod maturity and days to first pod maturity. No significant differences ($p > 0.05$) were observed in F_1 and RF_1 progenies suggesting the absence of maternal effect on the inheritance of extra-early maturity in cowpea.

Broad sense heritability varied from 75% to 99% while narrow sense heritability was 74% to 99%, indicating the importance of both additive and non additive variances implying that selection for improvement in the F_2 will be effective in improving early maturity in cowpea. The observed ratio (3:1) for F_2 and BC_1 indicating the inheritance of extra-early maturity in cowpea is dominant or partial dominant over late maturity and therefore influenced by monogenic dominance and recessive epistasis. Negative heterosis over mid-parent observed for early maturity parameters indicates the inheritance of early maturity was towards the extra-early parent (Sanzi). The additivedominance model revealed that both additive and non-additive gene effects contributed significantly to the inheritance of the trait studied suggesting the potential for further improvement through hybridization and selection procedures. Seed coat colour pattern was maternally inherited and various segregation colour patterns were observed in the F_2 and RF_2 . The seed coat colour pattern observed in the segregation populations could not fit in any of the modification in Medelian ratios suggesting the trait is quantitatively inherited.

List of abbreviations

CSIR	-	Council for Scientific and Industrial Research
CRI	-	Crop Research Institute
DAP	-	Days After Planting
DFF	-	days to 50% flowering
DFFI	-	days to first flower initiation
DNPM	-	days 90% pod maturity
DFPM	-	days to first pod maturity
FCDP	-	Food Crops Development Project
FAOSTAT	-	Food and Agricultural Organization Statistics
ICRISAT	-	International Crops Research Institute for the Semi-Arid Tropics
IITA	-	International Institute of Tropical Agriculture
MoFA	-	Ministry of Food and Agriculture
NASP	-	National Service Personnel
PBT	-	Plant Breeding Tools
SAR I	-	Savanna Agricultural Research Institute
WAAPP	-	West Africa Agriculture Productivity Programme

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cowpea (*Vigna unguiculata* (L.) Walp), a member of the Phaseoleae tribe of the Leguminosae family, is one of the most important versatile and nutritive grain legume crops native to Africa (Sivakumar *et al.*, 2013; Francisco *et al.*, 2014). The crop exhibits different morphological forms such as erect, semi-erect, climbing, prostrate or creeping and usually indeterminate under favourable environmental conditions (Timko *et al.*, 2007). The annual world cowpea production area was estimated at 11.8 to 14 million ha with an annual production of 4.5 to 5.4 million tons of dried grain and an average potential yield of 1.5 to 6 MT per ha (Singh *et al.*, 2002; FAOSTAT, 2010). Africa alone accounts for about 91% of the global production; West Africa, with 10.7 million ha, represents 75% of Africa's production (FAOSTAT, 2008). The principal cowpea producing countries are Nigeria, Niger, Brazil, Senegal, Mali, Burkina Faso and Ghana (FAOSTAT, 2000), with Nigeria, Niger and Mali leading the production in Africa (FAOSTAT, 2008). In West and Central Africa, cowpea is usually cultivated by subsistence farmers (usually women) on small scale as intercrop, in rotations or relay cropping with cereals such as sorghum, millet, and maize (Carlos, 2004).

Cowpea is cultivated in all the agro-ecological zones of Ghana based on local preferences for yield, maturity period, and grain size/colour (MOFA, 2010). Moreover, the bulk of the cowpea production in Ghana is largely found in the Guinea Savanna and

Forest Transition zones (CRI, 2006; Quaye *et al.*, 2011a).

The crop plays a very important role in achieving food security due to its high nutritional content of 23-30% protein, 50-67% carbohydrate, 1.9% fat, 6.35% fiber and small percentage of the B-vitamins such as folic acid, thiamine, riboflavin as well as some

micronutrients (Iron, Phosphorus, Zinc and Calcium) that improve human nutrition and health status (Bressani, 1985; Chinma *et al.*, 2008; Sefa-Dedeh *et al.*, 2011). Cowpea is being considered as a healthy alternative to soya bean as consumers look for more traditional food sources that are low in fat and high in fiber, and that have other health benefits (Moore and Ming, 2008). Protein from cowpea grain has good functional properties, including solubility, emulsifying and foaming activities and could be a substitute for soya bean for persons (especially infants) with soya bean protein allergies (Moore and Ming, 2008; Rangel *et al.* 2004). Processed food products of dry cowpea grain, such as cowpea-fortified baked foods, extruded snack foods, and weaning foods, have been developed to reduce malnutrition among children in Africa (Phillips *et al.*, 2003). According to ICRISAT (2012) report, malnutrition and infant mortality are expected to drop significantly through increased consumption of cowpea from the current level of 9 kg per capita to 15 kg per capita by most households in Ghana. The dry haulms of cowpea are used as fodder for livestock particularly during the dry season when animal feed is scarce making the crop an essential and integral part of sustainable crop-livestock farming systems in Sub-Saharan Africa (Blade *et al.*, 1997; Ortiz and Crouch, 2001; Langyintuo *et al.*, 2003; FAOSTAT, 2013). Cowpea is used in crop rotation, intercropping and as cover crop or green manure in relay cropping with cereals (Asibuo and Bonsu, 2000; Ennin and Cleggy, 2001). Moreover, cowpea is a shadetolerant crop, hence compatible as an intercrop with a number of cereals and root/tuber crops, as well as cotton, sugarcane and most plantation crops (Aveling, 1999;

FAOSTAT, 2013).

In addition, it grows quickly and permits establishment of a good ground cover and therefore improves the cropping systems and soil fertility by suppressing weed and reducing soil erosion (Duke, 1981). Cowpea cultivation plays a very significant role in sustainable farming system in Ghana due to its nitrogen fixing ability (Ennin-Kwabiah and

Osei-Bonsu, 1993; Quaye *et al.*, 2009). It fixes nitrogen up to 240 kg/ha and leaves about 60 –70 kg for succeeding crops (FCDP, 2005), and therefore contributes to increased yields of nitrogen demanding crops grown in rotation with it on the poor soils of Sub-Saharan Africa (Tarawali *et al.*, 2003).

It is obvious that, the importance of cowpea in the farming systems and as nutritious diet for millions of people and livestock makes it an ideal crop for achieving Millennium Developmental Goals of reducing poverty and hunger, improving human health and nutrition, and enhancing ecosystem resilience.

1.2 Problem statement

In Sub Saharan Africa, cowpea suffers considerable damage due to frequent terminal drought (as a result of climate change) especially during the pod filling stage (Agbicodo, 2009; Armah, 2010). Singh (1986) reported that early maturing varieties escape terminal drought and hence increases production and productivity.

Improving cowpea against constraints without farmer/consumer preference may result in the rejection of such varieties by farmers since farmer/consumer choice is very significant in utilization of cowpea in Ghana and the world at large (Egbadzor *et al.*, 2014). “Asontem”, is the most popular early maturing cowpea variety released by CSIR for cultivation in Ghana. However, its limitation for adoption by farmers is its red seed coat colour (Egbadzor, *et al.*, 2013) and it's poor performance in Sudan savanna agroecological zone (SARI, 2013).

Grain quality and yield of cowpea have been dramatically improved through traditional breeding strategies for the past few decades; however, reports of heritability estimates for extra-early maturity in the crop are rare (Nicole *et al.*, 2009).

1.3 Justification

To achieve food security and poverty reduction among resource-poor small holder farmers, an adaptive and strategic research of cowpea remains necessary; especially to breed for the best suited varieties for farmers (Van Duivenbooden *et al.*, 2002). The varietal requirements in terms of plant morphology, seed type, cropping system, maturity period are extremely diverse from one agro-ecological zone to another; this makes cowpea improvement programme more complex than for other crops (Singh *et al.*, 1997a). These varying preferences show the need to develop varieties with different characteristics, as no single variety can be suitable for all agro-ecological zones (Mashi *et al.*, 2006).

Extra-early maturing cowpea varieties can provide first food from the current harvest sooner than any other crop (in as few as 55 days after planting), thereby shortening the hunger period that often occurs just prior to harvest of the current season crops in farming communities in Sub Saharan Africa (Nicole *et al.*, 2009). According to Alpha *et al.* (2006) farmers in Savanna regions of Sub-Saharan Africa, adopt extra-early maturing varieties because they provided food security during the period of food scarcity in August/September; the emphasis is on earliness of crop maturity rather than on yield. Pswarayi and Vivek (2007) also reported that in areas where two cropping seasons occur, extra-early maturing crops provide additional seed for the main season cropping. The type of gene action involved in the expression of a trait is vital in deciding the appropriate breeding procedures to be used for the improvement of that trait (Johnson *et al.*, 1955; Adeyanju and Ishiyaku, 2007). Heritability estimate is a significant parameter in crop improvement programmes since it indicates how much of the phenotypic variability can be transmitted to the next generation (Falconer, 1981). It also suggests the extent to which crop improvement is possible through selection (Akhshi *et al.*, 2014). Therefore, if the genetic basis of extra-early maturity in cowpea is understood, it can be

exploited in the development of extra-early maturing cowpea varieties that can be cultivated in the changing climate and thereby ensure whole year availability of cowpea for the teeming population in Sub-Saharan Africa and the world at large. This research work seeks to investigate the genetic basis of extra-early maturity in cowpea by incorporating extra-early maturity genes from a land race (Sanzi) into locally adapted improved medium maturity cowpea cultivar (Padi-Tuya).

1.4 Objectives

The main objective of the study was to investigate the mode of inheritance of extra-early maturity in cowpea.

The specific objectives

- i. To estimate the heritability in extra-early maturity in cowpea.
- ii. To determine the type of gene action influencing the trait.
- iii. To determine the contribution of maternal effects on inheritance of extra-early maturity in cowpea.

CHAPTER LITERATURE REVIEW

2.1 History, origin and domestication of cowpea

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most ancient crops known to man and has been used as a crop plant since Neolithic times (Summerfield *et al.*, 1974). Cowpea was an important source of hay for cows in the southeastern United States and in other parts of the world and hence its name (Timko *et al.*, 2007). Its origin and subsequent domestication is closely linked with pearl millet and sorghum in Africa (World Cowpea Conference, 2010). Inadequate archaeological evidence has resulted in contradicting opinions supporting Africa, Asia, and South America as the center of origin of cowpea (Johnson, 1970; Summerfield *et al.*, 1974; Coetzee, 1995). The precise location of the origin and where cowpea was first domesticated is still under speculation (Ng, 1995).

Allen (1983) thought that cowpea was introduced from Africa to the Indian subcontinent about 2000 to 3500 years ago. On the other hand Ng and Padulosi (1998) revealed that before 300 BC, cowpea had reached Europe and possibly North Africa from Asia. They believe that, in the 17th century AD the Spanish took the crop to West India, and the slave trade from West Africa resulted in the crop reaching the southern USA early in the 18th

century. The centre of maximum diversity of domesticated *Vigna unguiculata* is found in West Africa, in an area within the Savanna regions (Ng, and Marechal, 1985). Interestingly, while West Africa appears to be the major center of diversity of cultivated cowpea (Ng and Padulosi, 1988) and was probably domesticated by farmers in this region (Ba *et al.*, 2004), the center of diversity of wild *Vigna* species is southeastern Africa (Singh *et al.*, 1997b).

Flight (1976) noted that carbon dating of wild cowpea remains from the Kintampo rock shelter in central Ghana revealed that, Kintampo is the oldest archaeological evidence of origin and domestication of cultivated cowpea. The archeological evidence shows the existence of gathering of cowpea by African hunters or food gatherers as early as 1500 BC.

Cultivated cowpea (sub sp.*unguiculata*) evolved through domestication and selection of the annual wild cowpea (var. *dekindtiana*). During the process of domestication and after the species was brought under cultivation through selection, there was a loss in seed dormancy and pod dehiscence, which resulted in an increase in pod and seed size.

(Harlan, 1992; Smith, 2006; Fuller, 2007).

2.2 Taxonomy of cowpea

Cowpea [*Vigna unguiculata* (L) Walp.] is a dicotyledonous crop in the order *Fabaceae*, subfamily *Faboideae* (Syn. *Papillionoideae*), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section *Catiang* (Verdcourt, 1970). The genus was divided into subgenera based upon morphological characteristics, the extent of genetic hybridization and geographical distribution of the species (Verdcourt, 1970). The major groups consist of the African sub-genera *Vigna* and *Haydonia*, the Asian sub-genus *Ceratotropis*, and the American sub-genera *Sigmoidotropis* and *Lasiopron* (Timko and Singh, 2008). *Vigna*

unguiculata sub-species *unguiculata* includes four cultivated groups: *unguiculata*, *biflora* (or *cylindrical*), *sesquipedalis*, and *textilis* (Ng and Maréchal, 1985). *Vigna unguiculata* subspecies *dekindiana*, *stenophylla*, and *tenuis* are intermediate wild progenitors of cultivated cowpea and form the major portion of the primary gene pool of cowpea, while wild subspecies like *pubescence* that do not readily hybridize and show some degree of pollen sterility form a secondary gene pool (Fatokun and Singh, 1987).

2.3 Cytology

According to Timko and Singh (2008), cowpea is a diploid plant containing 22 chromosomes ($2n=2x=22$) and its nuclear genome size is approximately 620 Mbp. The same nuclear genome size was found by Darlington and Wylie (1955); Faris (1964) and Frahm-Lelived (1965). According to Rachie and Roberts (1974), some cowpea varieties and their closely related weedy and wild relatives have $2n= 24$ chromosome number. However $2n=22$ is the more common condition.

2.4 Morphology and biology

Cowpea consist of diverse growth habits varying from erect, semi-erect, shrubby, trailing, prostrate, to climbing depending mostly on genotype, although photoperiod and growing conditions can also affect plant morphology (Timko *et al.*, 2007). These attributes of growth are generally due to genetic factors but may be also influenced by crop density, soil fertility, water stress, and the interaction of genotypes with day length and night temperatures (Steele and Mehra, 1980). Summerfield *et al.* (1974); Kay (1979) and Fox and Young (1982) described cowpea as an annual crop reaching heights of up to 80 cm with a strong tap root and many spreading lateral roots in the surface soil. Germination is epigeal, but cotyledons do not persist and may lose as much as 90% of their dry matter by the time seedlings emerge (Steele and Mehra, 1980). At the seedling stage, the first leaves

above the cotyledons are simple and opposite. Subsequent leaves are alternate and trifoliate with the terminal leaflet often bigger and longer than the two asymmetrical laterals. Leaflets are 5-18 cm long, 3-16 cm wide and are described as linear, lanceolate, or broadly or narrowly ovate, entire or obscurely toothed, broadly cuneate or rounded at the base and gradually tapering to a pointed tip. The petiole is stout, grooved, and 5-25 cm long. The stems are striate, smooth or slightly hairy and sometimes tinged with purple. The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles (Duke, 1981; Purseglove, 1984).

The nature of the peduncle is a distinguishing feature of cowpea, and this characteristic also facilitates hand harvesting (Kay, 1979; Fox and Young, 1982). Pods may be erect, crescent-shaped or coiled. They are usually yellow when ripe, but may also be brown or purple in colour. There are accessions with determinate and others with indeterminate growth habit. Pod length ranges from 4 cm in the wild subspecies to more than 1 m in subsp. *sesquipedalis*. Most cultivated species produce usually non-dehiscent, brittle or soft, curved, straight or coiled, and pendant, often constricted and distinctly beaked pods 12-20 cm long with about 10-15 seeds per pod.

Colour varies from brown, red or black to variously mottled with anthocyanic pigment (Steele and Mehra, 1980). Pods of wild species are straight, scabrous, slightly pubescent, black, erect or dehiscent. Cowpea seeds have diverse shapes, texture and colours. They are 2-12 mm long, kidney-shaped, oblong or cylindrical. The seeds may also be smooth or wrinkled, red, mottled, black, brown, green, buff or white as dominant full coloured, spotted, marbled, speckled, eyed, or blotched (Duke, 1981; Timko *et al.*, 2007; Timko and Singh, 2008). The weight of 100 seeds varies from 1 g in some wild species to 34 g in cultivars (Steele and Mehra, 1980).

Cowpea is highly self-pollinated crop in most production environments although significant out-crossing associated with insect activities can occur in some environments (Ehlers and Hall, 1997).

2.4.1 Floral characteristic

Cowpea is self-pollinated crop and pollination is complete before anthesis. Cross pollination seldom occurs, usually less than 1% depending on the cultivar and the pollinators (Blackhurst and Miller, 1980).

Flowers are borne on racemose inflorescences at the end of the pedicles that arise from leaf axils. Each unit is simple raceme with 6 to 12 flower buds (Ojehomon, 1968a). They are usually large with straight keel, and yellowish-white to purple colour. Each flower consist of 10 stamens, 1 free and 9 fused. They open in the early day and close at approximately midday. After blooming (opening once) they wilt and collapse (James and Robert, 2002, Summerfield *et al*; 1974). The style ends with an oblique stigma with becomes receptive before anthesis. There is high rate of abortion in cowpea; it can abort about 70 to 80% of its 100 to 500 flower buds prior to the opening of the flower. About half of the remaining may abort prematurely under certain environmental conditions, so that only 6 to 16% of the total flower buds produce pods (Ojehomon, 1968b). A low temperature regime promotes the pollination of cleistogamous flowers such as cowpea; pollen shed depends mainly on temperature, under warmer conditions (30°C or higher) pollen shed may occur before 8 am (Schuster, 1985).

2.5 Importance of cowpea

Cowpea plays a significant role in the livelihood of millions of people in Africa and other parts of the developing world where it is a major source of dietary protein that nutritionally

complements low-protein staples like cereal and tuber crops (SARI, 1996 and 1997). It is a very important and dependable crop that produces income for farmers and traders (Langyintuo *et al.*, 2003).

Fresh young leaves, immature pods are used as vegetables, while dry grain is used to prepare main meal dishes, snacks and canning (Quin, 1997; Davis *et al.*, 1991). It is a major source of vegetable protein (23-30%) and carbohydrates (64%). It is often referred to as poor man's meat (Bressani, 1985). It contains minerals like calcium and iron and amino acids (lysine, tryptophan, methionine) which improve human nutrition and health status (Adu-Dapaa *et al.*, 2005). It is the main source of protein for vegetarians (Narasinga, 1995). In Ghana, cowpea is an important source of vegetable protein and minerals for over 70% of the populace (MOFA, 2008). It is currently a food security crop in Ghana (MOFA, 2010).

In many areas around the world, cowpea is used for high quality leguminous hay as livestock feed particularly during the dry season when animal feed is scarce, making the crop an essential and integral part of sustainable crop-livestock farming systems in the semi-arid and arid regions of Sub-Saharan Africa (Ortiz and Crouch, 2001). On dry weight basis, the price of cowpea haulms ranges between 50 and 80% of the grain price, and therefore, haulms constitute an important source of income. The crude protein content ranges from 13 to 17% in cowpea haulms with high digestibility and low fiber and as a result, cowpea fodder is a good protein supplement to cereal stalks for sustainable livestock production (Tarawali *et al.*, 2000).

Cowpea is a valuable component of farming systems in many areas because of its ability to fix nitrogen for succeeding cereal crops grown in rotation with it (Sanginga *et al.*, 2003). It is also used as a green manure crop or for erosion control due to its rapid establishment and rapid ground cover (Davis *et al.*, 1991).

2.6 World production of cowpea

An estimated 14.5 million hectares of land is planted to cowpea each year worldwide, with the global production of 5.5 million metric tons of dried cowpeas grain in 2010 (FAOSTAT, 2012). Africa alone accounts for 91% of the world production. Nigeria is the largest producer and consumer (with per capita consumption of 25-30 kg) of cowpea, producing 2.2 million metric tons of dried grain in 2010. Nigeria is responsible for 61% of production in Africa and 58% of production worldwide with about 5 million ha and over 2 million tons annual production (FAOSTAT, 2012). Niger is the second largest producer, followed by Brazil, Burkina Faso, Myanmar, Cameroon, and Mali (Guazzelli, 1988; ICRISAT, 2011).

Niger Republic produces about 3 million ha and over 650, 000 tons production. Northeast Brazil grows about 1.5 million ha of cowpea with approximately 491, 558 tons production that provides food to about 25 million people. In Brazil as a whole, per capita consumption of cowpea is about 20 kg. In southern USA, about 40, 000 ha of cowpea is grown with an estimated 45, 000 tons annual production of dry cowpea seed and a large amount of frozen green cowpeas. India and Bangladesh are the largest cowpea producers in Asia (Singh *et al.*, 1997).

Millions of African farmers grow cowpea and the majority of these farmers are women who engage in subsistence cropping (Langyintuo *et al.*, 2003). ICRISAT 2011 reported that an estimated 38 million households (194 million people) grow cowpea in subSaharan Africa

2.6 .1 Cowpea production in Ghana

Cowpea is second to groundnut in terms of area under cultivation, quantity produced and consumed annually (Egbadzor *et al.*, 2013). An average of 143,000 MT is produced annually on about 156,000 ha making Ghana the fifth highest producer of cowpea in

Africa (ICRISAT, 2012). Ghana has the fastest growing production of the crop in Africa. Annual rates of growth for cowpea per area, yield and production for the period from 1985-1987 to 2005- 2007 were 0.1%, 39.6%, and 39.8%, respectively (ICRISAT, 2012). MOFA (2010) has projected that the rate of growth of cowpea production for the period between 2010 and 2020 would be 11.1%.

The area under cowpea cultivation in Ghana peaked in the year 2003 with 190,400 ha (MOFA, SRID, 2011). Subsequently, there have been slight reductions in the area under cowpea cultivation to 163,700 ha in 2010. However, the total cowpea grain production per annum has increased from 142,300 MT in 2004 to 219,300 MT in 2010 (Egbadzor *et al.*, 2013), with the Guinea Savanna zone of Ghana being the major production area in the country (Al-Hassan and Diao, 2007). Upper West Region and Northern Region produced 75,969 and 105, 841 MT, respectively, in 2010 (MOFA, SRID, 2011). Other production areas include Sudan Savanna zone (Upper East Region) and some Districts in the Transitional zone (Brong Ahafo Region). Unfortunately, production can be done only within a short period in the year in these regions due to the long period of drought (Langyintuo *et al.*, 2003).

As a result the domestic production of cowpea cannot meet the national requirements and a considerable portion of cowpea grain is imported to supplement its demand (Quaye *et al.*, 2011a). According to Langyituo (1999); Seferiadis (2000); Langyituo *et al.* (2003), Ghana imports 10, 000 MT of cowpea annually; 30% from Burkina Faso and 70% from

Nigeria. There is a huge production and consumption gap which can be reduced by breeding improved cultivars desired by farmers (Azam *et al.*, 2013).

2.7 Climatic and soil requirements

Comparably, cowpea is known to have good adaptation to high temperatures and drought stress (Padi, *et al.*, 2004). Cowpea is a tropical crop which requires less rainfall than most crops; hence the bulk of its production is in the dry savannah regions. Heavy rainfall encourages excessive vegetative growth and disease incidence is higher (SARI, 2012). Cowpea can be grown under rain-fed conditions as well as by using irrigation or residual moisture along river or lake flood plains during the dry season. Cowpea performs well in agro-ecological zones where the rainfall range is between 500 and 1200 mm/year (Madamba *et al.*, 2006). If irrigation is used, more vegetative growth and sometimes delay in maturity may result. Application rates should ensure that the crop is not over-watered, especially in more northern latitudes, as this will suppress growth by lowering soil temperatures. The most critical moisture requiring period is just prior to and during bloom. (Davis *et al.*, 1991)

It germinates rapidly when temperature is above 19°C, and requires minimum and maximum temperatures of between 28 and 30°C during the growing period (Craufurd *et al.*, 1996).

Cowpea is well adapted to sandy and poor soils; on heavy fertile soils they show vigorous vegetative growth, but not necessarily good grain yield. The best yields are obtained in well-drained sandy loam to clay loam soils with the pH between 6 and 7 (Dugje *et al.*, 2009).

Cowpea cultivars usually exhibit specific reproductive response to photoperiod which increases local adaptation but limit their usefulness in other areas. Cowpea developed for one region therefore may not perform well in other regions (Padi *et al.*, 2004).

2.8 Production constraints

Cowpea yields are generally low in Sub-Saharan Africa, due to several biotic and abiotic constraints coupled with cultivation of cowpea as an intercrop with cereals in marginal environments, where soils are infertile and rainfall is scanty (Ram *et al.*, 2005). Under intercropping, the growing cereals shade cowpea, and therefore compete with them for growth resources such as moisture, sunlight and nutrients, and cause severe reduction in cowpea yields. Most farmers in Africa cultivate cowpea without insect pest protection measures, which lead to poor growth and severe yield reduction due to pests damage (Singh, 2005; Timko *et al.*; 2007). These constraints can be categorized into biotic and abiotic constraints.

2.8.1 Biotic constraints

2.8.1.1 Pest

Insect pests are a major constraint to cowpea production (Rusoke and Rubaihayo, 1994), because each phase attracts a number of insect pests. Many insect pests and parasitic weeds attack cowpea. Parasitic weeds such *Striga gesnerioides* and *Alectra vogelli* are most common yield reducers in cowpea in Africa (Tsekenedza, 2013; Parker and Richens, 1993). Both weeds are difficult to control because they produce large number of seeds, and up to 75% of the crop damage is done before they emerge from the soil; but *Striga* is more devastating than *Alectra* (Ram *et al.*, 2005; Dugje *et al.*, 2009). Abunyewa and Padi, (2003) indicated that, in the Sudan savannah zone of Ghana striga infestation is very

significant and that, an average number of 9,384 seeds m⁻² was found in the land that had been recultivated after fallow.

The major insect pests of cowpea are aphids, (*Aphis caccivora*), thrips (*Magalurothrips sjostedti*), Maruca (*Maruca vitrata*), a complex of pod sucking bugs (*Megaralla spp.*, *Acanthonia spp.*, *Riptortus spp.*) and the storage weevil (*Callosobruchus maculatus*) they can cause up to 100% loss to cowpea grain if not controlled (Ezueh, 1981). In SubSaharan Africa thrips, aphids and maruca are the major field insect pests of economic importance to the crop (Ram *et al.*, 2005).

2.8.1.2 Disease of cowpea

Cowpea is susceptible to a wide range of diseases at all stages of its growth cycle (Allen, 1983). Some of these are cowpea wilt caused by *Fusarium oesclporium*, cowpea root rust caused by a nematode (*Meloidogyne ssp*), Aphid-borne mosaic virus, cowpea bacterial blight caused by *Xanthomonas vignicola* and stem rot caused by *Phytophthora vignae*. Losses due to diseases can be as high as 90% (International Institute of Tropical Agriculture, IITA, 2000).

2.8.2.1 Abiotic constraints

Drought is a major abiotic stress that limits crop performance more especially in drier savanna and sahelien regions, where it significantly influences plant performance and survival and for that matter leads to constraints in plant functioning, including a series of morphological, physiological and metabolic changes (Ludlow and Muchow, 1990). Seed production, which is positively correlated with leaf area (Rawson and Turner, 1982), can be reduced by drought-induced stress. However, the early maturing cowpea cultivars tend to be very sensitive to drought that occurs during the early stages of the reproductive phase

(Thiaw and Parker, 1993). Eighty-five percent of the world's cowpea production is concentrated in the savanna zone of West Africa, which is located between 10° and 20° N latitude (FAOSTAT, 1972). Droughts occur frequently in this area, most commonly due to erratic start or early cessation of rainfall during the growing season, or occasionally, due to almost no rainfall during the normal growing season (Wien *et al.*, 1976).

Though cowpea is inherently more drought tolerant than other food crops, it still suffers considerable damage due to frequent drought in the regions where rainfall is scanty and irregular. (Ram *et al.*, 2005) The increased incidence of drought in some cowpea growing areas has caused a shift to early maturing varieties (Mortimore *et al.*, 1997). Early maturity in cowpea cultivars is desirable and has proven to be useful in some dry environments because of the ability of such cultivars to escape terminal drought (Hall and Patel 1985; Singh, 1994) and, pest and disease damage that normally occur later in the cropping season (Kauret *et al.*, 2009). Such early cultivars can reach maturity in as few as 55 DAP in many of the cowpea production ecological zones of Africa.

2. 9 Importance of extra-early maturing cowpea varieties

Singh *et al.* (2007) and Dugje *et al.* (2009) classified cowpea varieties that mature in less than 60 DAP as extra- early, 61-75 DAP as early and more than 80 DAP as late.

Farmers' preference for extra-early and early maturing cowpea cultivars in Sub-Saharan Africa is similar to other regions in the world and has been well documented (Singh *et al.*, 2007).

In efforts to cope with rainfall risk in Sub-Saharan Africa, many small-scale farmers purposefully pursue multiple planting dates over extended periods of time in order to avoid total crop failure (Rorhrbach, 1998). Pswarayi and Vivek (2007) reported that, farmers grow early maturing crop varieties because such varieties provide an early harvest to

bridge the hunger period before harvest of a full season crop. In Savanna regions of Sub-Saharan Africa, farmers adopt extra-early maturing varieties because they provide food security during the period of food scarcity in August/September; the emphasis is on earliness of crop maturity rather than on yield (Alpha *et al.*, 2006). Extraearly maturing varieties are ideal for offseason plantings in drying riverbeds; they are also suitable for intercropping as they provide less competition for growth resources than the late maturing varieties (CIMMYT, 2000; FAOSTAT, 2013).

Singh *et al.* (1997) noted that extra-early varieties have opened the possibility of successful sole cropping in areas with short rainy season, double/triple cropping in areas with relatively longer rainfall, and relay cropping after millet, sorghum or maize as well as intercropping with cereals and root and tubers.

2.10 Heritability

Heritability is the proportion of observed phenotypic variability of a trait among individuals of a given population that are due to genetic differences, and this is what determines the degree of resemblance between relatives (Falconer, 1960). Factors such as genetics, environment and random chance can all contribute to the phenotypic variation among individuals in a population. It is a significant parameter in crop improvement programme, because it indicates how much of the phenotypic variability can be transmitted to the next generation (Falconer, 1981). The magnitude of such estimates also suggests the extent to which crop improvement is possible through selection (Akhshi *et al.*, 2014). For parents to transmit characteristics to their offspring in some predictable degree, it is obvious that environmental variance should be low and genetic variance high (Strickberger, 1976). According to Sivakumar *et al.* (2013), the variability between individuals in a population is the sum total of heritable and nonheritable components; and

a high value of heritability indicates that the phenotype of that trait strongly reflects its genotype.

The objective of crop improvement is to create new gene combinations and useful variability among genotypes by intercrossing parents that possess desirable characteristics or by introducing new germplasm from other breeding programs. This variability is then narrowed by selection of the few genotypes that perform best in the target environment (Bänziger, 2000). Falconer (1989) observed that breeders make the most selection progress when:

- Genetic variance among genotypes is large.
- Selection intensity is high; thus only a small proportion of genotypes are selected.
- Heritability is high; that is, traits that are valuable in the target environment can be assessed precisely in the genotypes evaluated and are transmitted to the offspring of these genotypes.

Inheritance of a character is very significant to a breeder because it provides him an idea of the extent of genetic control for the expression of a particular trait (Chopra, 2000). Heritability is an important parameter in breeding program. It indicates how much of the phenotypic variability can be transmitted to the next generation (Falconer, 1981). The magnitude of such estimates also suggests the extent to which improvement is possible through selection. (Falconer, 1981). Furthermore, heritability serves as a guide to the reliability of phenotypic variability in the selection programme and hence determines its success (Hamdi, 1992). Nausherwan *et al.* (2008) reported that polygenic variation may be phenotypic, genotypic or environmental and relative values of these three types of coefficients give an idea about the magnitude of the variability.

Broad senses heritability refers to the ratio of heritable variance to total variance [$h^2b = V_G/V_P$]. In a narrow sense, heritability is defined as the ratio of additive genetic

variance to total variance [$h^2n=V_A/V_P$]. Narrow sense heritability is important to plant breeder because the effectiveness of selection depends on the additive variance in relation to phenotypic variance (Falconer, 1960). Moreover, heritability in the broad sense is the result of the sum of the additive effect and the dominance deviation which is broken in the next generation due to the independent segregation of the alleles (Hugo *et al.*, 2014). According to Ubi *et al.* (2001), heritability estimates along with genetic advance are more useful in predicting the resultant effect for the selection of the best individual from a population. High narrow sense heritability values indicate the predominance of additive gene action in the expression of traits and can be improved through individual plant selection (Makeem *et al.*, 2007; Rashwan, 2010). Ayo-Vaughan *et al.* (2011) reported that earliness in cowpea broad sense heritability estimate was high (99%) for both days to flowering and to maturity evaluated, but narrow-sense heritability estimate was low (1.8% for days to maturity; 2.0% for days to flowering).

Broad sense heritability tends to yield a high value and for that matter, the narrow sense heritability estimate is more useful to plant breeders than the broad sense estimate (Aquaah, 2007). Strickberger, (1976) also stated that, the additive proportion (V_A) of phenotypic variance is of greater importance in heritability than dominant proportion (V_D), because of this, narrow sense heritability is generally used as a measure of inheritance or heritability of traits. Broad sense heritability has a narrow inference space to plant breeders because, it highly depends on the genetic differences between the two particular inbred lines used (Nicole *et al.*, 2009). Therefore, heritability estimated from the cross of two inbred lines cannot be generalized to other populations or line crosses. A simple method commonly used to estimate trait heritability in cowpea is to measure the phenotypic variance among P_1 , P_2 , F_1 , BC_1 , BC_2 and F_2 individuals developed from the

cross between two inbred lines. The total phenotypic variance among the F_2 consists of the genetic variance and the environmental variance. The environmental variance can be estimated by the average of the phenotypic variance among plants of the parental lines and the F_1 (Nicole, *et al.*, 2009).

2.10.1 Heterosis

Acquaah (2007) defined heterosis in two basic ways: better-parent heterosis and midparent heterosis. Better-parent heterosis is calculated as the degree by which the F_1 mean exceeds the better parent in the cross. Mid-parent heterosis is defined as the superiority of the F_1 over the means of the parents. The most important development in plant breeding of recent times is the extensive use of heterosis (Malik *et al.*, 1987).

Heterosis depends on the non-additive gene action. The estimate of heterosis % over mid-parent and inbreeding depression studied by Abd-Elhady (2003) and Zaveri *et al.* (1983) found that Heterosis (%) over mid-parent ranged from -0.48% for days to flowering to 22.2% for weight of seeds/plant. Inbreeding depression ranged from 22.01% for weight of seeds/plant to 4.07% for days to flowering. Falconer and Mackay 1996 reported that heterosis can only occur when parental cultivars used for F_1 development differ in gene frequencies.

2.10.2 Maternal effect

Quantitative geneticists have historically defined maternal effects as the influence of the maternally provided environment on the phenotype of her offspring (Dickerson, 1947; Willham, 1972; Legates, 1972; Cheverud, 1984). In this phenomenon the contribution of the maternal parent to the phenotype of its offspring is beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987). Maternal effects occur

when an organism shows the phenotype expected from the genotype of the mother, irrespective of its own genotype, often due to the mother supplying mRNA or proteins to the embryo; it is associated with the inheritance of quantitative and qualitative traits and therefore may affect responses to selection (Etterson and Galloway, 2002). Maternal effects arise from egg cytoplasm which has been modified by chromosomally transmitted genes (Strickberger, 1976). Its distinguishing characteristic is the difference in the results of reciprocal crosses,

The genotype of the mother via maternal effect account for a considerable portion of the genetically based variation in progeny phenotype of many traits. Hence, selection based on direct genetic effect may not be adequate, and may lead to omission of potentially important source of genetic variance contributed by the cytoplasm of the maternal strain (Wolf *et al.*, 2002). Mothers often provide much of the environment for their offspring. These maternal effects are predicted to result in unusual evolutionary dynamics in offspring traits if they are themselves heritable (McAdam and Boutin, 2003). A mother can influence a trait in her offspring both by the genes she transmits (*Mendelian inheritance*) and by maternal attributes that directly affect that trait in the offspring (maternal effect). Maternal inheritance can alter the direction, rate and duration of adaptive evolution from standard Mendelian models and its impact on adaptive evolution has not been adequately explored in natural populations (Thiede, 1998). According to Dickerson (1947), the importance of maternal effects has long been recognized by quantitative geneticists, although they have largely regarded them as non-genetic environmental sources of resemblance of relatives (Falconer and Mackay, 1996; Futuyma, 1998) and a nuisance that contaminates estimates of heritability (Wade, 1998).

2.10.3 Gene action

Generation mean analysis is a simple but useful technique for estimating gene effects for a polygenic trait, its greatest merit lying in the ability to estimate epistatic gene effects such as additive \times additive, dominance \times dominance and additive \times dominance effects (Singh and Singh, 1992). Besides gene effects, breeders would also like to know how much of the variation in a crop is genetic and to what extent this variation is heritable, because efficiency of selection mainly depends on additive genetic variance, influence of the environment and interaction between genotype and environment. (Singh and Singh, 1992). Gene action is very important in the study of quantitative traits because it deals with the way genes express themselves. It is divided into additive and non-additive effects. Nonadditive gene action is again sub- divided into dominance and epistasis (Robinson *et al.*, 1949; Falconer 1989). In the presence of additive gene action, characters of the heterozygotes in the F_1 generations are the intermediate of the two parents (Falconer, 1989). According to Falconer, (1989) the additive gene effect reflects the degree to which progenies are likely to resemble their parents and non-additive gene action is observed when the additive model cannot adequately explain the variation. The study conducted by Robinson *et al.* (1949) pointed out that, the size of dominance relative to the additive variance indicates the degree of dominance, which can be a range of partial to over-dominance in relation to the mean of their parents. Ishiyaku *et al.* (2005) reported that additive (a) and additive \times dominance (d) interactions were the most important gene actions conditioning time to flowering with a narrow sense heritability of 86% estimated for this trait. They concluded that time to flowering in cowpea is controlled by at least seven major gene pairs.

2.10.4 Inheritance of early maturity in cowpea

Early maturity is an important agronomic trait, it is a significant component of adaptation of crops to any agro-ecological zone especially in the semi-arid tropics, where it is associated with some stress factors that occur late in the growing season (Ayo-Vaughan, 2011; Singh, 1985). It is measured by such criteria, as days to first flower with corolla visible, days to 50% flowering and days to maturity (Adeyanju and Ishiyaku, 2007).

According to Brittingham, (1950), early maturity is dominant or partially dominant over late maturity. However, Capinpin and Irabagon, (1950) indicated that late maturity is dominant over early maturity, and (Adeyanju and Ishiyaku, 2007; Ojomo, 1971) thought that duplicate dominant epistasis between two major genes in the presence of some minor modifying genes are responsible for early maturity in cowpea. Brittingham (1950) speculated that the maturity is inherited quantitatively.

A number of quantitative studies of the genetics of early maturity parameters such as broad-sense heritability estimates averaged 48.3 % days for flowering and 47.8% for days to pod maturity (Mak and Yap, 1980). Several authors concluded that additive genes action is responsible for much of the genetic variation for early maturity of cowpea (Tikka *et al.*, 1977; Mak and Yap, 1980; Zaveri *et al.*, 1983). Dumbre *et al.*, (1983) reported broad sense heritability estimates of 52% and 42% for maturity and pod filling period respectively. Duplicate dominant epistasis between two major genes in the presence of some modifying genes is responsible for the inheritance of days to first flower and much of the genetic variation for days to flowering is due to dominance or epistasis (Ojomo, 1971).

The study conducted by Bastian *et al.*, (2000) on the inheritance mechanism for number of days to first flower and to maturity in intra-specific crosses of *Vigna unguiculata* (L) Walp showed that additive, non additive, additive with partial dominance and over

dominance genetic effects were responsible in the expression of the traits. Ayo-Vaughan *et al.* (2011) revealed that both additive and non-additive gene action are involved in the control of early maturity inheritance in cowpea. Tuba and Sakar, (2008) noted that, days to flowering was regulated by gene effects that was additive in nature, and days to maturity was predominantly regulated by additive and dominance gene actions.

2.11 Inheritance of seed coat color

Mann (1914) revealed that anthocyanin and a melanin-like substance are responsible for colour in plants and the expression of any pigment on the plant is the result of the interaction between several pigment genes and a general colour factor. Seed coat colour pigmentation may also be influenced by environmental factors such as solar radiation (Egbadzor *et al.*, 2014).

The genetics of seed coat colour in cowpea has not yet been understood due to the interactions and modifier genes that control the trait (Fery, 1980). Spillman in (1912) postulated that a general color factor C, is responsible for color and its absence results in white seeds. The C factor in combination with R, U, Br, Br and N, and N and B conditions red, buff, brown, black, and blue seed coat, respectively. On the other hand, Harland (1919) proposed a model with R as a general color factor conditioning red seed coat. He stated that the R factor with B, N, M, and N and M conditions black, buff, maroon, and brown, respectively.

Spillman and Sando (1980) designated the general color factor as R and described N as an anthocyanin pigment factor. They used symbols B, F, P and U for brown, fine and dense speckling, purple, and buff, respectively, and showed how these genes interacted to produce 10 different seed coat colours. According to Saunders (1960), colour patterns of the cowpea seed coat result from interactions between two or more genes. This suggests

that seed coat colour in cowpea is quantitatively inherited. He stated that the gene responsible for black color is dominant to all but the purple seed color. However, Calub (1968) indicated that black is epistatic to all colors regardless of the presence of other colour genes. Seed coat patterns are inherited independently of seed coat colour of the parental genotypes, but the appearance of any pattern depends upon the presence of the general colour factor C (Calub, 1968; Fery, 1980). Spillman and Sando (1980) proposed five genes controlling various seed coat patterns while Fery (1980), suggested that several of the genes governing the trait may be allelic. Due to incomplete dominance of seed coat colour pattern genes, classification is difficult in segregating progeny for the Holstein, Watson, small eye, and hilum ring traits (Drabo *et al.*, 1988).

2.12 Flowering

Flowering is an important physiological process in crop survival and assurance for its continuity. Time of flowering is particularly of great importance in annual crops, including cowpea, as it is a component of the adaptation of a variety to a particular agroecological zone and it also determines pod set, crop yield and maturity period (Ishiyaku *et al.*, 2005). Plant growth and development, especially flowering, is dependent on the interaction of many complex processes which are influenced by both genetic and environmental factors (Uarrota, 2010). Diepenbrock (2000) found that, the onset of flower initiation can have strong influence on flower, pod and seed number. Moreover, timing of flowering determines when crops ripen for harvest (Ayo-Vaughan *et al.*, 2011). According to Craufurd *et al.* (1996) and Mukhtar and Singh (2006), photoperiod is the most vital environmental variable affecting time of flowering in cowpea in West and Central Africa since most varieties under cultivation are unimproved local types which are photoperiod

sensitive. Photoperiod has been reported to influence plant growth characteristics, including flowering.

Photoperiod can be defined as the developmental responses of plants to the relative length of light and dark periods. Many flowering plants use photoreceptor proteins, such as phytochrome or cryptochrome in various degrees and for that matter are classified as day neutral, long day and short day plants (Mauseth, 2003). According to Singh (1993) cowpea genotypes whose days to first flowering is greater than 45 are photoperiod sensitive (long or short day) while those that flower in less than 45 days are photoperiodinsensitive or day neutral. Plant height, leaf length, leaf area and growth habit as well as flowering are highly regulated by photoperiod (Cha-um and Chalermopol 2007). Ojehomon (1967) reported that short days during the growing season limit the growth and flowering in cowpeas. In the short day varieties, the plants are short and erect but when grown under long-day conditions (>13 hrs), the plants grow bushy, become prostrate and may delay flowering or may not flower at all. Similarly, cowpea plants growing under long days grow taller and appear more vigorous with broader and greener leaves than those under normal days (Doku, 1969). Ojehomon (1968b) observed that long photoperiods caused delay flowering and abscission of flower buds in cowpea.

2.13 Artificial hybridization

Artificial hybridization between parental genotypes is the first step to initiate segregating populations for breeding varieties. Cowpea flower is cleistogamous, with self-pollination occurring shortly before anthesis (Asiwe, 2009). Though cowpea is a highly selfpollinated crop, for genetic improvement purpose artificial cross pollination is very necessary and its success has been reported to range from 0.5 to 50% (Rachie *et al.*, 1975). This range varies with genetic and physiological factors as well as the technical expertise in handling the

floral parts during the emasculation process. A low temperature regime promotes the formation of cleistogamous flowers, pollen shed depends mainly on temperature, under warmer conditions (30°C or higher) pollen shed may occur before 8-9 am (Schuster, 1985) which does not favour artificial hybridization and the growing of parental material in a growth chamber or greenhouse is recommended (Gridnev and Kochegura, 1988).

The first crossing between crop wild relatives and cultivars to obtain disease resistant varieties date back to the 1890's (Rawal, 1975), Several reports (Ng and Marechal, 1985; Ng, 1990; Mohammed *et al.*, 2010) indicated that wild and the weedy subspecies of cowpea (*V. unguiculata* subsp. *dekindtiana*, *stenophylla*) hybridize easily with the cultivated forms and produce viable and heterotic hybrids. However, according to Rawal *et al.* (1976), the wild form could only be used as the male parent and attempts to use it as the female parent were unsuccessful. To successfully use wild relatives of cowpea effectively for cultivar improvement, their cross compatibility and reproductive potential need be ascertained (Nwosu and Awa, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the experiment

The research was conducted at the CSIR-Savanna Agricultural Research Institute (CSIRSARI), Tamale. SARI is in the Guinea Savanna agro-ecological zone of Ghana, located on latitude 9⁰, 25', 41N and longitude 0⁰, 58', 42W and about 183 m above sea level. The rainfall is monomodal with an average annual rainfall of about 1200 mm. The rains begin in May and end in October. The cropping season therefore, starts in mid-June to October with the rest of the year being dry and hazy (Agro metrology section-SARI, 2012). The soil is well drained Nyakpala series classified by FAO as Ferric Luvisol. It is brown, fine sandy loam, with low organic matter. (William-SARI, Personal communication)

3.2 Experimental Material

Two genotypes of cowpea namely, Padi-Tuya and Sanzi obtained from CSIR-Savanna Agricultural Research Institute were used for the study (Fig 1).

Padi-Tuya is an elite variety released by SARI in 2008 for general cultivation to increase cowpea production and productivity in the Savanna ecology of Ghana. However, it has gained popularity in transitional agro-ecological zone of Ghana particularly Ejura, due to its attractive attributes such as high yield, large grain size, and cream seed coat colour with black eye. It has indeterminate and erect growth habit, and matures between 70-75 days after planting (SARI, 2012).

Sanzi (a landrace) is tolerant to drought and most pests and diseases of improved cultivars of cowpea and matures within 45-50 days (SARI, 2012). It has determinate and spread growth habit, with small and mottled grain.



Figure 1 Experimental material

3.3 Methodology

Three experiments were conducted between May, 2014 and May, 2015 as indicated below.

The first and the second experiments which made up of artificial hybridization and backcrossing respectively were carried out in pots and on prepared beds from May, 2014 to October, 2014. The third experiment was conducted in the field from March, 2015 to May, 2015 under drip irrigation at CSIR-SARI Technology Park.

3.3.1 Experiment one: Development of F₁ seeds

Thirty plastic pots of diameter 20 cm base, 30 cm top and 35 cm height were used to plant each of the parental genotypes Padi-Tuya and Sanzi. Planting was staggered with the early maturing variety (Sanzi) 10 days later than the medium maturing variety (PadiTuya) for synchronous flowering. Agronomic practices were followed to raise healthy crop.

At flowering, female flowers (fully matured non-opened) were emasculated by using forceps. Pollen grains from opened male flowers were placed on the stigma of emasculated female flower before 7:00 am. Each cross was tagged and labeled for easy identification, and at maturity, the F₁ pods were harvested separately and dried. Direct and reciprocal crosses were made to produce F₁ seeds and their reciprocals as indicated below;

Padi-Tuya (♀) × Sanzi (♂) → F₁

Sanzi (♀) × Padi-Tuya (♂) → RF₁

3.3.2 Experiment two: development of F₂, RF₂ and backcross (BC₁, BC₂, RBC₁ and RBC₂) generations.

The parental genotypes, the F₁ and RF₁ were stager-planted on pots. The parental genotypes were backcrossed to their respective F₁ and RF₁ to obtain BC₁, BC₂, RBC₁ and

RBC₂. At the same time, F₁ and RF₁ were selfed to produce F₂ and RF₂ respectively as indicated below.

Table 1: Developed backcrosses and second filial generations

Parents	Genotypes
1. F ₁ (♀) × Padi-tuya (♂)	Backcross one (BC ₁)
2. F ₁ (♀) × Sanzi (♂)	Backcross two (BC ₂)
3. RF ₁ (♀) × Padi-Tuya(♂)	Reciprocal backcross one (RBC ₁)
4. RF ₁ (♀) × Sanzi (♂)	Reciprocal backcross two (RBC ₂)
5. F ₁ (selfed)	F ₂
6. RF ₁ (selfed)	F ₂ reciprocal (RF ₂)

3.3.3 Experiment three; Field evaluation

The experimental material for field evaluation comprised 10 genotypes, generated from the above crossed and selfed combinations. These genotypes were planted in the field in triplicate, using Randomized Complete Block Design (RCBD), for evaluation at CSIRSavanna Agricultural Research Institute Technology Park. The experiment was conducted under drip-irrigation during dry season (6th March – 28th May, 2015).

The plot size comprised two rows each for non-segregating generations (P₁, P₂, F₁ and RF₁), 3 rows each for BC₁, BC₂, RBC₁ and RBC₂ generations and 10 rows each for F₂ generations. Each row (constituting 10 plants) was 5 m long with row spacing of 1 m and a distance of 40 cm between plants within row. The sample size (number of plants analyzed) varied as follows: 60 plants for the P₁, P₂ and F₁ and RF₁ generations, 300 plants for the F₂ and RF₂ generations and 90 plants in the BC₁, BC₂, RBC₁ and RBC₂ generations. One seed was planted per hill, refilling was done immediately after emergence. Starter dose of 2 g NPK 15:15:15 was applied 14 DAP. Field pests were controlled using K-Optimum at the rate of 1.5 litres per hectare at vegetative, flowering and at podding. Weeding was done manually when necessary.

Treatment	Genotypes	Pedigree	Rep.1	Rep. 2	Rep. 3
T1	Padi-Tuya	SARC 3-122-2	109	206	304
T2	Sanzi	Landrace	110	207	301
T3	1 st Filial Generation (F ₁)	Padi-Tuya x Sanzi	104	205	306
T4	1 st Filial Generation (RF ₁)	Sanzi x Padi-Tuya	108	202	309
T5	Backcross 1(BC ₁)	F ₁ x Padi-Tuya	103	208	305
T6	Backcross 2 (BC ₂)	F ₁ x Sanzi	106	204	302
T7	Recip. Backcross 1 (RBC ₁)	RF ₁ x Padi-Tuya	101	201	308
T8	Recip. Backcross 2. (RBC ₂)	RF ₁ x Sanzi	107	210	303
T9	2 nd Filial Generation (F ₂)	Padi-Tuya x Sanzi (Selfed)	105	203	307
T10	Reciprocal 2 nd Filial Generation (RF ₂)	Sanzi x Padi-Tuya (Selfed)	102	209	310

Table 2. Field randomization of experimental material.

3.4 Parameters measured

3.4.1 Mean percentage seedling emergence

The number of seeds germinated from each treatment was counted a week after planting and the number expressed as percentage of the total number of seeds planted.

3.4.2 Mean days to 1st flower

This was recorded as the number of days from sowing to the first flower on a plant for each population on each plot.

3.4.3 Mean days to 50% flowering

Days after sowing until half the plant population of each plot have one or more flowers.

3.4.4 Mean plant height at flowering

At flowering, plant height was measured in centimeters on all the plants on each plot, taking from the base of the plant to the last node on the main stem.

3.4.5 Mean plant height at maturity

Plant height was measured in centimeters on all the plants per plot, taking from the base of the plant to the last node on the main stem when 90% of the pods on each plot matured.

3.4.6 Mean days to 90% pod maturity

This was recorded as the number of days from sowing till 90% of the pods on each plot matured.

3.4.7 Mean number of peduncle

Number of peduncle per plant was counted and recorded for each plot.

3.4.8 Mean 100 seed weight (g)

Hundred seeds from the plants in each plot were weighed and recorded in grams.

3.4.9 Seed coat colour

Seeds harvested from F₂ and RF₂ were grouped into various colours for each generation to determine the segregation ratios. The segregation ratios obtained were subjected to chi-square test to determine the goodness of fit to the various genetic ratios.

3. 5 Statistical and genetic analyses

Data for all the variables measured were subjected to analysis of variance (ANOVA), to estimate the level of variability and significant differences between generation means among the cowpea accessions, using GENSTAT version 12 software. Where the difference was significant ($p < 0.05$) treatment means were separated using least significant difference (LSD) test at 5%.

The following statistics were also estimated using GENSTAT version 12 software on the parents, F₁, F₂, BC₁ and BC₂ populations for each of the crosses: variance, means, standard deviation, standard error and coefficient of variation.

3.5.1 Heritability estimate

Variance components (additive, dominance, environment, genetic and phenotypic) were estimated as described by (Wright, 1968; Aquaah, 2007), using the following equations:

$$V_A = 2V_{F_2} - (V_{B_1} + V_{B_2})$$

$$V_D = [(V_{B_1} + V_{B_2}) - F_2 - (V_{P_1} + V_{P_2} + F_1)]/3$$

$$V_E = [V_{P_1} + V_{P_2} + V_{F_1}]/3$$

$$V_G = V_A + V_D + V_I$$

$$V_P(V_{F2}) = V_G + V_E + V_{GE}$$

Broad sense heritability = $h^2_b = V_G/V_P$, while narrow sense heritability = $h^2_n = V_A/V_P$

(Allard, 1960; Warner, 1952). Where, V_A = additive variance, V_D = dominance variance, V_E = environmental variance, V_G = Genetic variance, V_P = phenotypic variance. While, parent 1 (V_{P1}), parent 2 (V_{P2}), first filial generation (V_{F1}), second filial generation (V_{F2}), backcross 1 (V_{BC1}) and backcross 2 (V_{BC2}) variances

3.5.2 Estimation of inheritance pattern for maturity

Based on the days to first flower initiation and days to first pod maturity, the F_2 segregation population was classified into extra-early and medium maturity. Singh *et al.*

(2007) and Dugje *et al.* (2009) classified cowpea varieties that mature in less than 60

DAP as extra- early, 60--70 DAP as early, 70-80 DAP as medium and more than 80 DAP as late. The chi-square test of significance was used to investigate gene interactions for the F_2 generations. The segregation ratios were analyzed through the χ^2 value which was obtained from the following formula:

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

3.5.3 Heterosis estimate

Heterosis (H%) was estimated as deviation of F_1 value from the mid-parent (MP) and from the better parent values (heterobeltiosis, HB%) as outlined by Fonseca and Paterson (1968):

$$H\% = [(F_1 - MP) / MP] \times 100$$

$$HB\% = [(F_1 - BP) / BP] \times 100. \text{ Where, } MP = (P_1 + P_2)/2 \text{ and } BP = \text{best parent.}$$

3.5.4 Estimation of Gene action

Gene effects based on six parameters (P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2) were estimated using the nonweighted generation means analysis described by Gamble (1962) with the help of Plant Breeding Tools Version 1.4 to test for the adequacy of the additive-dominance model (PBT, 2014).

3.5.5 Estimation of maternal effects

The maternal effects were investigated by comparing the mean values of F_1 with the mean values of RF_1 (reciprocal F_1) using a mean-difference test at 5% level of significance (Steel and Torrie, 1991).

3.6 Determination of seed coat colour pattern

The seeds from F_2 and RF_2 were grouped separately according to the various seed coat colour patterns. Data obtained from the inheritance of seed coat colour being a qualitative trait, was subjected to Chi-square analysis to test for the goodness of fit to the proposed segregation ratio.

CHAPTER FOUR

4.0 RESULTS

4.1 Mean days to 50% flowering

The result showed that the mean for the 10 cowpea genotypes exhibited highly significant ($P < 0.01$) differences for days to 50% flowering. The means, standard errors, variances and co-efficient of variation for days to 50% flowering, of the progenies of Padi-Tuya \times Sanzi and their reciprocals are presented in (Tables 3). It was noted that Sanzi had significantly lower days to 50% flowering of 31.3 whiles Padi-Tuya had 44.67. There was no significant ($P > 0.05$) differences between F_1 (36.00) and RF_1 (36.33) for the trait. No significant ($P > 0.05$) differences were also observed between BC_1 (41.00) and BC_2 (39.00) and for RBC_1 (39.33) and RBC_2 (38.60). There was no significant difference in the variance of the segregation and none segregation populations.

4.2 Mean days to first flower initiation

Similarly, highly significant ($P < 0.01$) differences were observed between Padi-Tuya (44.60) and Sanzi (32.03) (Table 3). On the other hand no significant ($P > 0.05$) differences were noted between F_1 (34.87) and RF_1 (35.07) for days to first flower initiation. These were also observed in BC_1 (40.23) and BC_2 (39.53) and also RBC_1 (39.60) and RBC_2 (38.97). The variance for none segregation populations (P_1 , P_2 , F_1 and RF_1) ranged between 2.99 and 6.59 whiles that of the segregation populations (F_2 , RF_2 , BC_1 , BC_2 , RBC_1 , RBC_2) ranged from 22.89 to 44.88.

4.3 Mean days to 90% pod maturity

Highly significant differences were observed in days to 90% pod maturity except each of the following F_1 and RF_1 , BC_1 and BC_2 , RBC_1 and RBC_2 in (Table 4). Sanzi had significantly lower days to 90% pod maturity of 49.33 whiles Padi-Tuya had 71.33. The

means of the F_1 (55.00) and RF_1 (54.67) for days to 90% pod maturity were less than their mid-parent value (60.33).

4.4 Mean days to first pod maturity

Similarly, highly significant differences were observed in days to first pod maturity except each of the following F_1 (50.00) and RF_1 (49.60), F_2 (56.80) and RF_2 (56.87), BC_1 (55.90) and BC_2 (54.17), RBC_1 (53.73) and RBC_2 (52.70) in (Table 4). The means of the F_1 (50.00) and RF_1 (49.60) for days to first pod maturity were less than their mid-parent values (53.56) for days to first pod maturity but closer to that of Sanzi (44.93). The F_2 segregating populations had larger variances among the genotypes.

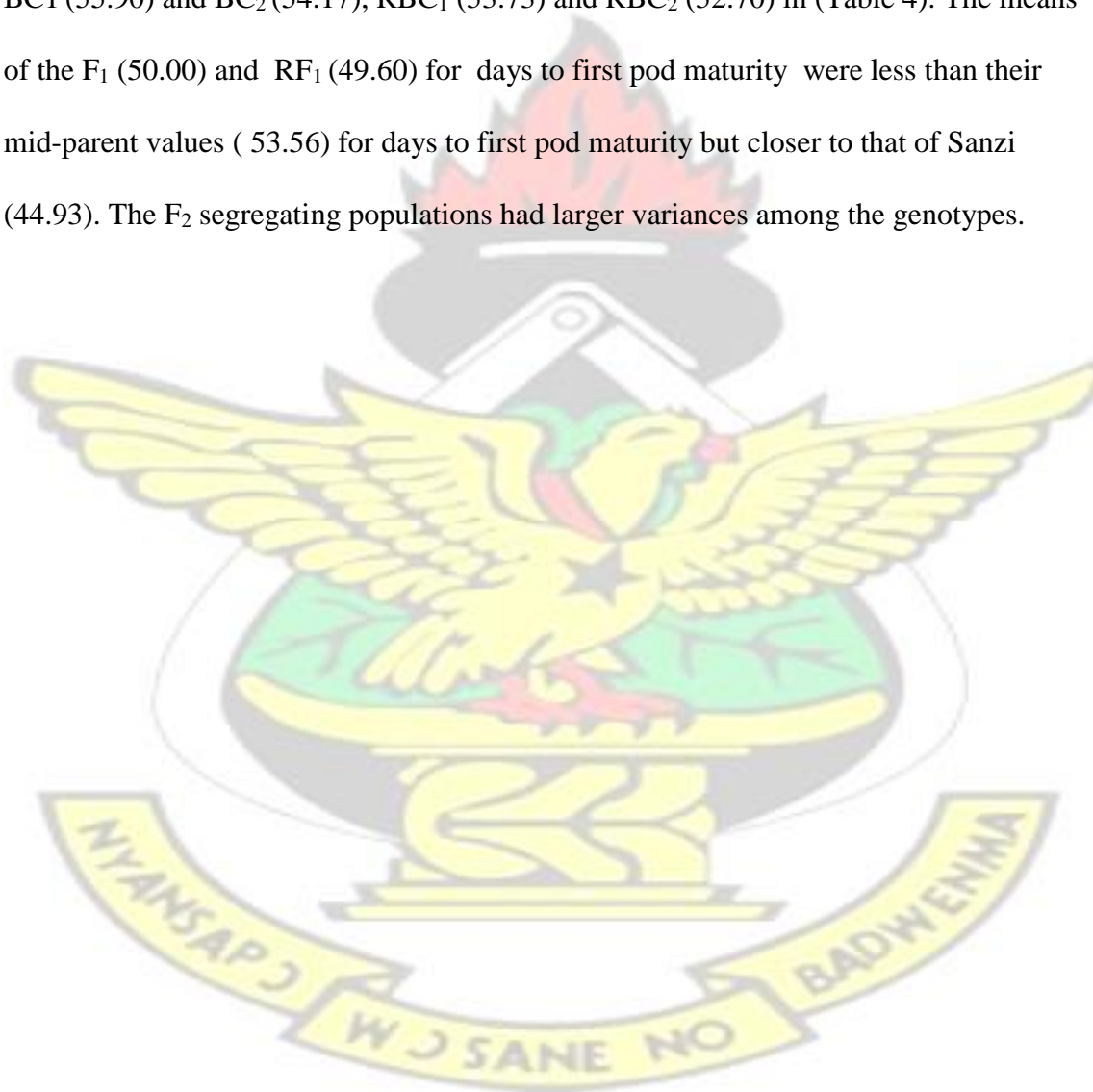


Table 3. The means, standard errors, variances, standard deviation and co-efficient of variation for days to 50% flowering (DFF) and days to first flower initiation (DFFI) for the two parents (Padu-Tuya and Sanzi) and their eight progenies obtained from direct and reciprocal crosses.

Generation	NO. of plants	DFF					DFFI				
		Mean	SE	S ²	SD	CV(%)	Mean	SE	S ²	SD	CV(%)
P1	60	44.67e	0.45	1.52	1.23	2.75	44.60f	0.82	6.59	2.57	5.06
P2	60	31.33a	0.80	1.88	1.37	4.37	32.03a	0.32	2.99	1.73	5.00
F ₁	60	36.00b	0.90	2.47	1.57	4.36	34.87b	0.46	4.35	2.10	5.10
RF ₁	60	36.33b	0.86	2.22	1.49	4.10	35.07b	0.46	3.30	1.82	5.00
F ₂ RF ₂	300	43.3de	0.60	2.89	1.70	3.93	42.23e	1.09	44.88	6.70	15.30
BC ₁	300	39.33bc	0.91	3.48	1.86	4.73	41.5de	0.91	44.52	6.67	16.29
BC ₂	90	41.00cd	0.99	2.98	1.73	4.22	40.23ce	1.11	28.19	5.31	12.05
RBC ₁	90	39.00bc	0.55	1.92	1.38	3.54	39.53cd	1.06	22.89	4.78	12.00
RBC ₂	90	39.33bc	0.78	2.26	1.50	3.81	39.60cd	0.53	37.01	6.09	14.95
MP	90	38		1.03	1.01	2.61	38.97c	0.54	33.82	5.81	14.99
BP		44.67					38.30				
LSD		3.34					44.60				
							2.18				

Means followed by the same letter(s) are not significant at $p < 0.05$

SE= Standard Error. MP = Mid-parent value, BP = Best-parent value, S² = Variance, SD = Standard deviation.

Table 4. The means, standard errors, variances, standard deviation and co-efficient of variation for days 90% pod maturity (DNPM) and days to first pod maturity (DFPM) for the two parents (Padu-Tuya and Sanzi) and their eight progenies obtained from direct and reciprocal crosses.

Generation	NO. of plants	DNPM					DFPM				
		Mean	SE	S ²	SD	CV (%)	Mean	SE	S ²	SD	CV(%)
P1	60	71.33e	1.18	4.18	2.03	2.86	62.20f	0.98	22.00	4.69	7.09
P2	60	49.33a	0.69	2.43	1.56	3.16	44.9a	1.14	19.36	4.40	10.10
F ₁	60	55.00b	0.84	2.14	1.46	2.65	50.00b	0.83	11.83	3.41	6.00
RF ₁	60	54.67b	0.50	2.75	1.66	3.03	49.60b	0.73	9.90	3.15	6.04
F ₂ RF ₂	300	72.33e	0.68	7.65	2.76	3.81	56.80e	0.55	69.63	8.34	14.20
BC ₁	300	71.00e	1.62	8.67	2.94	4.14	56.87e	0.52	52.30	7.24	12.00
BC ₂	90	61.00d	0.87	4.39	2.09	3.43	55.90de	0.75	42.82	6.54	11.12
RBC ₁	90	58.33cd	1.47	3.90	1.97	3.37	54.17cd	0.66	45.20	6.72	12.00
RBC ₂	90	59.67cd	0.47	2.31	1.52	2.55	53.73c	0.66	38.32	6.19	11.00
RBC ₂	90	57.00bc	1.24	6.52	2.55	4.48	52.70c	0.79	79.07	8.89	16.09
BP	71.33						62.20				
H%		-8.50					-6.65				
LSD (5%)	2.90						2.87				

Means followed by the same letter(s) are not significantly different at $p > 0.05$.

SE= Standard Error. MP = Mid-parent value, BP = Best-parent value, S^2 = Variance, SD = Standard deviation.



4.5 Variance components and Heritability

Table 5 shows the genotypic, phenotypic, environmental and additive variances for days to 50% flowering (DFF); days to first flower initiation (DFFI); days to 90% pod maturity (DNPM) and days to first pod maturity (DFPM), obtained for the six generations (P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2). Generally, the phenotypic variance was higher than the genotypic variance in all the traits studied. The magnitude of the genotypic variance was however higher than the environmental variance (Table 5). The values of phenotypic variances ranged from 4.89 (DFF) to 69.03 (DFPM), genotypic variances varied from 4.84 (DFF) to 51.90 (DFPM), environmental variance varied from 1.96 (DFF) to 17.73 (DFPM), additive variance ranged from 4.84 (DFF) to 51.24 (DFPM), while dominance variances also ranged from -1.95 (0) (DFF) to 1.56 (DFFI).

Estimated heritability values were very high and varied slightly between parameters studied (Table 5). Broad-sense heritability (h^2_b) ranged from 74.50 (DFPM) to 99% (DFF), and narrow-sense heritability (h^2_n) varied from 73.60% (DFPM) to 99% (DFF).

4.6 Degree of dominance

Table 5 also indicates the degree of dominance (d) for days to 50% flowering (0%); days to first flower initiation (28%); days to 90% pod maturity (0%) and days to first pod maturity (16%)

Table 5. Estimates of genetic parameters based upon variances of days to 50% flowering (DFF); days to first flower initiation (DFFI); days to 90% pod maturity (DNPM); days to first pod maturity (DFPM)

PARAMETER	DFF	DFFI	DNPM	DFPM
Phenotypic variance	4.89	44.88	7.65	69.63
Environmental Variance	1.96	4.64	2.92	17.73
Genotypic variance	4.84	40.24	7.01	51.90
Additive variance	4.84	38.68	7.01	51.24
Dominance variance	-1.95 (0)	1.56	-2.27 (0)	0.66
Broad sense Heritability (%)	99	89.7	91.6	74.5
Narrow sense Heritability (%)	99	86.2	91.6	73.6
Degree of Dominance (%)	0	28	0	16

4.7 Determination of inheritance pattern in maturity

Number of plants for days to first flower initiation (DFFI) and days to first pod maturity (DFPM) are presented in (Table 6). All the 60 plants of Padi-Tuya (P1) fell within the medium maturity range (70-80 DAP) while the 60 plants of Sanzi (P2) were in extraearly maturity category (< 60DAP). On the other hand, 227 plants of F₂ generation fell within extra-early maturity and 79 plants were in medium category. In BC₁ segregation population, 49 plants were extra-early and 41 plants were categorized into medium maturity.

Table 6. Number of plants expressing days to first flower initiation (DFFI) and days to first pod maturation (DFPM).

Generation	No of plants studied	No. of plants/DFFI		No. of plants/DFPM	
		Extraearly/Early	Medium	Extraearly/Early	Medium
P1	60	0	60	60	0
P2	60	60	0	60	0
F ₁	60	60	0	60	0
F ₂	300	221	79	228	72
BC ₁	90	48	42	47	43

4.8 Segregation patterns for Extra-early and Medium.

Segregation ratios, chi-square and P values for extra-early and medium in days first flower initiation and days to first pod maturity at F₂ and BC₁ are presented in Tables 7 and 7.1. The phenotypic ratios; 3:1 for F₂ and 1:1 for BC₁ were used to test for goodness of fit of observed segregation at F₂ and BC₁ using the Chi-square test. Calculated chisquare values were less than the P values in both traits (Tables 7 and 7.1).

Table 7 Segregation pattern for days to first flower initiation (DFFI) in F₂ and BC₁ progenies of Padi-Tuya and Sanzi

		No. of plants/DFFI						
Gen.	No. of plants studied	Extra-early		Medium		Ratio	Chi- square	P Value
		Observe	Expected	Observe	Expected			
F ₂	300	221	225	79	75	3:1	0.28	0.59
BC ₁	90	48	45	42	45	1:1	0.40	0.53

Table 7. 1 Segregation pattern for days to first pod maturity (DFPM) in F₂ and BC₁ progenies of Padi-Tuya and Sanzi.

No. of plants/DFPM								
Gen.	No. of plants studied	Extra-early		Medium		Ratio	Chisquare	P Value
		Observe	Expected	Observe	Expected			
F ₂	300	228	225	72	75	3:1	0.16	0.68
BC ₁	90	47	45	43	45	1:1	0.18	0.67

4.9 Heterosis

Table 8 presents means of traits studied for parental lines and their F₁ hybrids. The F₁s means values were less than mid-parent and best parent values. Both mid-parent heterosis (H%) and better-parent heterobeltiosis (HB%) varied among each of the traits. Thus Days to 50% flowering (-5.26, -19.46); days to first flower initiation (-8.95,-21.82); days to 90% pod maturity (-22,-22.00); days to first pod maturity (-6.64, -21.82) for midparent (heterosis) and better parent (heterobeltiosis) respectively.

Table 8 : Average Performance of Parental lines and F1 Generations, and Heterosis estimates for Days to 50% flowering (DFF); days to first flower initiation (DFFI); days to 90% pod maturity (DNPM); days to first pod maturity (DFPM).

Accession	DFF	DFFI	DNPM	DFPM
P ₁ (Padi-Tuya)	44.67	44.60	71.33	62.20
P ₂ (Sanzi)	31.33	32.03	49.33	44.93
F ₁	36.00	34.87	55.20	50.00
Mid-parent	38.00	38.31	60.33	53.56
Best-parent	44.67	44.60	71.33	62.20
H%(Heterosis)	-5.26	-8.85	-8.50	-6.65
HB%(Heterobeltiosis)	-19.41	-21.82	-22.61	-19.61

4.10 Generation mean analysis

The results from the analysis of variance in (Tables 3 and 4) revealed a significant difference among the generations for all investigated traits in the cross, indicating the existence of genetic variation. Hence, generation mean was analyzed to estimate the genetic components for all the traits studied.

Different types of genetic components are presented in (Tables 9 and 9.1). The estimated mean effect component (m) was found to be highly significant ($P < 0.01$) for all traits studied except for days to first pod maturity which was not significant ($P > 0.05$). The additive (a) gene action was positive and highly significant ($P < 0.01$) for days to 50% flowering, days to first flower and days to first pod maturity.

The estimated dominance (d) gene action was negative but highly significant for days to first flower initiation ($P < 0.01$) and also significant for days to first pod maturity ($P < 0.05$).

However, it was not significant for days to 50% flowering and days to 90% pod maturity ($P>0.05$).

A highly significant ($P<0.01$) negative additive x additive (*aa*) epistasis gene action was recorded for days to first flower initiation and significant ($P<0.05$) for days to first pod maturity. But it was not significant ($P>0.05$) for days to 50% flowering and days to 90% pod maturity.

The additive x dominance (*ad*) gene effect was negative and not significant ($P>0.05$) for days to 50% flowering, days 90% pod maturity but significant ($P<0.05$) in days to first pod maturity. Generally, additive gene action was the major genetic component of the inheritance of the traits studied.

Table 9. Gene effect parameters for days to 50% flowering and days to first flower initiation

Genetic component	Estimate	Std. Error	t-value	Pr(> t)
Days to 50% flowering				
m	46.42**	1.69	27.34	0.02
a	6.62*	0.51	13.07	0.05
d	-9.96 ^{ns}	2.47	-4.02	0.15
aa	-8.17 ^{ns}	1.78	-4.59	0.14
ad	-13.98 ^{ns}	2.58	-5.41	0.15
χ^2 (2 df)	1.293 ^{ns}			
Days first flower initiation				
m	49.91**	0.47	106.31	0.01
a	6.30**	0.18	34.19	0.02
d	-14.87**	0.58	-25.60	0.02
aa	-11.56*	0.51	-22.65	0.03
ad	-11.27 ^{ns}	1.23	-9.19	0.06
χ^2 (2 df)	0.17 ^{ns}			

**= Significant at 0.01. *= Significant at 0.05. χ^2 = Chi-square. df= degree of freedom.

Table 9.1 Gene effects parameters for days to 50% pod maturity and days to first pod maturity

Genetic component	Estimate	Std. Error	t-value	Pr(> t)
Days to 90% pod maturity				
m	96.20*	5.93	16.22	0.04
a	10.20 _{ns}	2.46	4.15	0.15
d	-14.75 _{ns}	8.63	-4.15	0.13
aa	-34.83 _{ns}	6.44	-5.40	0.12
ad	-14.01 _{ns}	14.65	-0.95	0.51
χ^2 (2 df)	12.45 _{ns}			
Days first pod maturity				
m	61.46**	0.36	167.69	0.001
a	10.46**	0.17	59.72	0.01
d	-10.26*	0.52	-19.66	0.03
aa	-6.34*	0.41	-15.41	0.04
ad	-15.09**	0.66	-22.78	0.02
χ^2 (2 df)	0.08 _{ns}			

**= Significant at 0.01. *= Significant at 0.05. χ^2 = Chi-square. df= degree of freedom.

4.11 Maternal effects

The result from the analysis of variance presented in (Tables 3 and 4) showed that there was no significant difference ($P>0.05$) between the mean values of F_1 and RF_1 in days to 50% flowering, days to first flower initiation, days to 90% pod maturity and days to first pod maturity.

4.12 Inheritance of seed coat colour pattern

Results for the direct and reciprocal crosses of Padi-Tuya and Sanzi are presented in (Figure 2). The F_1 and RF_1 , progenies inherited the seed coat colour of their respective maternal parents cream and mottled with black eyes as illustrated below.



Figure 2: Direct and reciprocal crosses of Padi-Tuya and Sanzi

F_2 and RF_2 plants on the other hand produced varied seed coat colours ranging from black to cream making it very difficult to classify (Figures 3 and 4). Interestingly, different seed coat colour patterns were observed in a number of the same plants and in the some of the pods. Twelve different groups of seed coat colours were identified in an attempt to group the seed, based on seed coat colour pattern for F_2 segregation population, whiles thirteen were observed in RF_2 . They produced solid, eye, and multicoloured seed coat colour patterns.



Figure 3: Segregation in F2 seed coat colour pattern (Sampled)

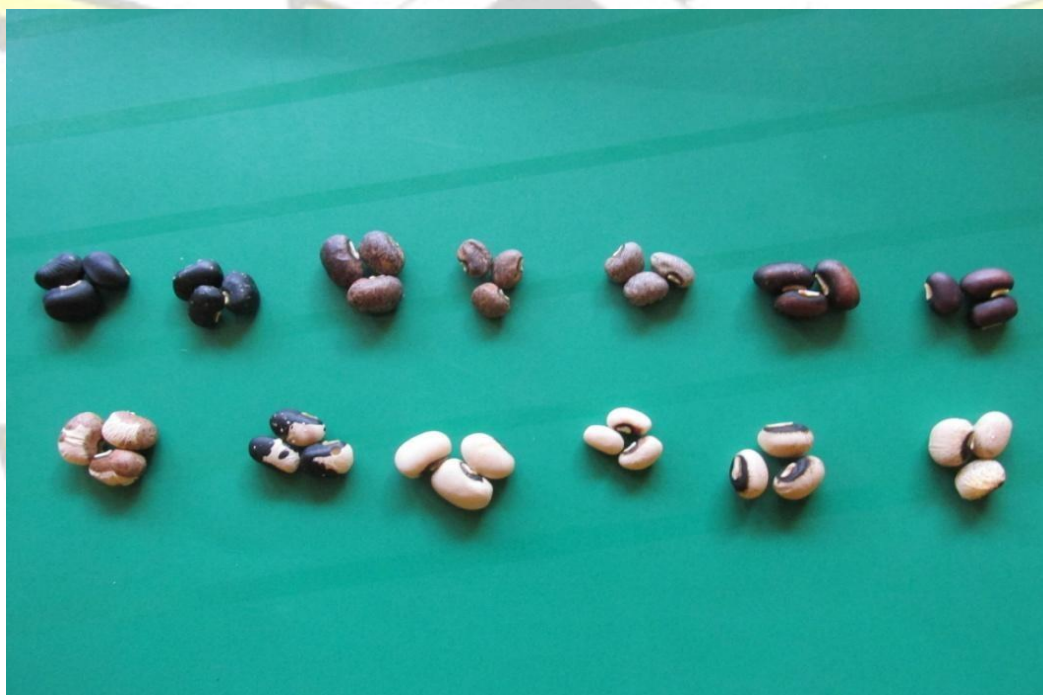


Figure 4: Segregation in RF2 seed coat colour pattern (Sampled)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Evaluation of early maturity

The means of days to 50% flowering, days to first flower initiation, days to 90% pod maturity and days to first pod maturity of the F_1 progeny were lower than their midparent mean values and closer to the early maturing parent (Sanzi). In a self-pollinated crop such as cowpea a departure of F_1 mean value from its mid-parent value indicates the effect of dominance or partial dominance. This agrees to the findings of Brittingham (1950) which stated that early maturity is dominant or partially dominant over late maturity. There was a highly significant difference ($p < 0.01$) between the parents. According to Mather and Jinks (1982), the phenotypic difference between the parental lines is of the utmost importance for inheritance studies, aiming to obtain the most precise estimates of genetic parameters. Backcross, F_2 and RF_2 populations of the cross combinations had a larger variance than the corresponding F_1 , RF_1 and the parents. This shows the phenomenon of F_2 and back cross segregating populations.

There were no significant differences ($P > 0.05$) between the means of BC_1 and BC_2 which implies in backcross breeding programme to improve early maturity in cowpea the choice of a recurrent parent is not important.

5.1.1 Variance components

The variance components for days to first flower initiation and days to first pod maturity were positive, except for variance due to dominance for days to 50% flowering and that of days to 90% pod maturity which were negative values and for practical purposes were considered as zero. Negative values for dominance and additive variances resulting in zero values were also observed in previous studies using segregating populations of cowpea by Hugo *et al.* (2014) and segregating populations of wheat by Bakarat (1996). Phenotypic variance was higher than genotypic variance suggesting the trait was quantitatively inherited. The values of genetic variance for all the studied parameters were greater than

those observed for the environmental variance. Suggesting that early maturity trait in cowpea can be improved through selection. However, days to first pod maturity recorded the highest environmental variance of 17.73, while days to first flower initiation recorded 4.64. These findings reveal that pod maturity is greatly influenced by environment than flowering. The additive variance was the most important genetic component of the traits evaluated than dominance variance resulting in high narrow sense heritability estimates. This indicates that additive effects were primarily responsible for the genetic variation in the traits examined. This finding suggests the possibility of early generation selection for improving early maturity of cowpea, Sakar and Bicer (2004) and Bicer and Sakar (2008) have already reported similar finding.

5. 2 Heritability

The study revealed that, the heritability estimates were high for all traits studied. High values of heritability indicate the phenotype of that trait strongly reflects its genotype (Sivakumar *et al.*, 2013). Broad sense heritability estimates varied from 74.5% to 99% for days to 50% flowering; days to first flower initiation; days to 90% pod maturity and days to first pod maturity, while narrow sense heritability also ranged from 73.6% to 99% for days to 50% flowering; days to first flower initiation; days to 90% pod maturity and days to first pod maturity suggesting the traits studied are highly heritable and selection for improvement in the early generation will be effective in improving early maturity in cowpea. Similar heritability estimates have been reported for days to flowering and days to pod maturity by Sharma and Singhania (1992); Adeyanju and Ishiyaku (2007); Suganthi and Murugan, (2008) and Sivakumar *et al.* (2013). On the other hand, Ayo-Vaughan *et al.* (2011) reported a high broad sense heritability of 99% and very low narrow sense heritability estimates of 1.8% for days to pod maturity and 2.0% for days to flowering. These contrasting findings have scientific basis because heritability highly depends on the

genetic differences between the two particular inbred lines used (Nicole *et al.*, 2009). Tweneboah (2000) also noted that, soils with high nitrogen may produce excessive vegetative growth and delay flowering which is undesirable for grain production in legumes such as cowpea. Cowpea is a drought tolerant legume, producing appreciable yield and early flowering under conditions of high temperatures/solar radiation and low rainfall, where other crops may fail to thrive (Doku and Karikari, 1969).

Therefore, heritability estimated on cowpea maturity from the cross of two inbred lines and on different agro-ecological zones or cropping seasons cannot be generalized to other populations or line crosses.

There was no significant difference between the broad and narrow sense heritability estimates in the traits studied. This was due to negative dominance variance which drastically reduced the broad sense heritability. However, heritability in the broad sense in self-pollinating crops is less informative than heritability in the narrow sense which is a direct measure of additive variance (Caviness, 1969; Strickberger, 1976; Tsuchiya, 1986). High estimates of narrow-sense heritability indicate that additive effects were primarily responsible for the genetic variation in the extra-early maturity of the cowpea genotypes evaluated.

Additive effect is very important in improving self-pollinated crops such as cowpea, since it does not segregate much from generation to generation, making it possible to successfully select in segregating populations (Warner, 1952). Hence, backcross, pedigree, single-seed descent methods are recommended for advancing such segregating populations (Bernado, 2003). Several authors noted that additive gene effect is responsible for much of the genetic variation for early maturity of cowpea (Tikka *et al.*, 1976; Mak and Yap, 1980; Zaveri *et al.*, 1983). A high heritability estimate of the trait also suggests that high selection pressure should be imposed in any breeding programme aimed at improving early maturity in cowpea (Acquaah, 2007).

The degree of dominance for all the early maturity indicators examined ranged from 0% - 28% indicating predominance of additive genetic effect in controlling these traits. This was also noted by Asadollah (2010) who studied selection effect, genetic advance and genetic parameters in rice. Even though some dominance effect may also occur, predominance of additive genetic effect means inheritance of extra-early maturity in cowpea can be improved by selection in early generation (Lopes *et al.*, 2013).

5.2 1. Determination of allelic relationship between inheritance of Extra-early and Medium cowpea genotypes.

The observed segregation frequency in the F₂ generation was 3 extra-early to 1 medium (3:1). While frequency observed in BC₁ generation was 1 extra-early to 1 medium (1:1). Calculated chi-square values were less than the P values in each case, which implies the inheritance of days to first flower initiation and days to first pod maturity conforms to the 3:1 and 1:1 ratios, and therefore we accept the null hypothesis. This further indicates the inheritance of extra-early maturity in cowpea is under monogenic dominant control.

Similar observations were made by Brittingham, (1950); Hugo *et al.* (2014) when he studied genetic parameters of earliness and plant architecture traits suitable for mechanical harvesting of cowpea (*Vigna unguiculata*) and Gatut *et al* (2014) in his study of mode of inheritance of genes control maturity in soybean (*Glycine max.* (L) Merrill).

The minimal gene number indicates that few selection cycles would be necessary to obtain the required accumulation of the favourable alleles controlling the inheritance of extra-early maturity in cowpea. Therefore the development of extra-early cowpea cultivars can be relatively simple through classical breeding strategies that are employed in self-pollinated crops.

5.3 Heterosis

The parameters studied had *negative heterosis/heterobeltosis*, which imply that the means of the hybrid fell below the mid-parent means with respect to each trait. Negative values of the traits (DFF, DFFI, DNPM and DFPM) are desirable, since early maturity is an important objective for this study. It also implies dominance or partial dominance gene action was responsible for inheritance of early maturity in cowpea since the mean of the F₁ was closer to the extra-early parent (Sanzi). Earlier study by Bello and Odunayo (2015) in maize had similar result for days to tasselling and cob maturity and thereby concluded that the early maturity in maize was conditioned by dominance or partial gene action.

5.4 Generation mean analysis

Calculated χ^2 value was found to be insignificant for all the parameters, as it indicates the adequacy of the additive-dominance model (Jawahar *et al.*, 2013).

Highly significance of parental mean effect (*m*) and additive effect (*a*) explained about 75% of the total variation of the traits in segregating generations of cowpea crosses (Matos Filho, 2006). These are in agreement with the genetic components estimated in this study and for that matter underlined the importance of the additive gene action in extra-early maturity parameters (DFFI, DNPM and DFPM) in cowpea. This finding suggests the potential for obtaining further improvement of extra-early maturity in cowpea by using single decent method. Similar findings were reported by Bhor and Dumber (1998), Abd-Elhady (2003) and Rashwan (2010).

Additive (*a*), gene effect was the most important gene action conditioning days to flowering and days to pod maturity with a narrow sense heritability of 86.20% and 73.6% respectively. Similar result was noted by Ishiyaku *et al.* (2005). Besides additive gene action, the significant differences in epistatic component of additive x additive (*aa*)

indicates the preponderance of additive over non-additive gene action. In such cases, to improve early maturity in cowpea, pedigree method will be rewarding (Akhshi, *et al.*, 2014). Such interactions have been noticed in all traits in the present study, thus days to 50% flowering, days to 90% pod maturity, days to first flower initiation and days to first pod maturity.

However, dominance gene action (d); (-14.87, - 10.26), additive \times additive (aa); (-11.56, - 6.34) and additive \times dominance (ad); (-11.27, -15.09) for days to flowering and days to maturity respectively also played a role in the inheritance of the extra-early maturity in the cowpea genotypes evaluated. Dominance gene action being negative suggests it was toward the extra-early parent (Sanzi). Additive \times additive (aa), additive \times dominance (ad) gene action being significant in both cases suggest additive \times additive and additive \times dominance epistasis also played an important role in the inheritance of the trait. According to Khattack *et al.* (2002), traits with additive \times additive (aa) type of epistasis can be exploited by standard hybridization and selection procedures.

There was no dominance \times dominance (dd) epistatic effect on the inheritance of the traits studied. In addition, the opposite signs of (a) and (d) for all the parameters suggest that duplicate type of epistasis played a role in days to first flower initiation, days to first pod maturity, days to 50% flowering and days to 90% pod maturity. This finding was in perfect agreement with that of Akhshi *et al.* (2014), who studied generation mean analysis to estimate genetic parameters for morphological traits in common bean (*Phaseolus vulgaris* L.). It also confirms reports by Ojomo, (1971) and Adeyanju and Ishiyaku (2007) which stated that duplicate epistasis between two major genes in the presence of some minor modifying genes are responsible for early maturity in cowpea. Inheritance of early maturity in cowpea is quantitatively inherited and hence under polygenic control. Brittingham, (1950) and Ishiyaku *et al.* (2005) had already pointed out

that maturity in cowpea is quantitatively inherited and therefore conditioned by at least seven major genes with other modifying genes.

5.5 Maternal effects

No significant differences ($P > 0.05$) was observed between F_1 ($P_1 \times P_2$) and RF_1 ($P_2 \times P_2$) in the parameters studied, suggesting the absence of maternal effects for inheritance of maturity in cowpea. The trait could therefore be attributed to nuclear gene control, and cytoplasmic genes had no effect on it. For that matter the choice of maternal parent is not important in hybridization programme that focuses on the improvement of maturity in cowpea. Absence of maternal effects has direct implications on the selection process and the progression of segregating populations in genetic improvement programme (Allard, 1960). For this reason, selection for inheritance of extra-early maturity in cowpea should begin in F_2 generation where ample variability was observed.

5.6 Seed coat colour pattern

The cross between Padi-Tuya (cream) and Sanzi (mottled) produced cream and mottled seeded F_1 and RF_1 progenies respectively. This implies the inheritance of seed coat colour pattern in cowpea is maternally influenced. Therefore the choice of maternal parent is very important in cowpea hybrid seed programme aimed at improving seed coat colour. The observation in this study contradicts that of Mustapha (2009) and Egbadzor (2014) which noted that, the cross between green and red seeds of cowpea produced brown seeded F_1 progeny and that of red and cream colour parents produced black seeded F_1 progeny respectively, an indication of incomplete dominance of the red seeded parent over the green. However, Ndambe (2005) reported maternal inheritance of cowpea seed colour pattern in his study of inheritance of antioxidant activity and its association with seed coat color in cowpea (*Vigna unguiculata* (L.) walp.). Nicole *et al.* (2009) noted that heritability

highly depends on the genetic differences between the two particular inbred lines used hence heritability estimated from the cross of two inbred lines cannot be generalized to other populations or line crosses. Sanzi (a landrace) might have an influence of the inheritance on seed coat colour as reported by Guei and Traore, (2001) that landraces are good sources of unique genes.

An attempt to group the distribution of seed coat colour pattern in the F_2 segregating population to some simple Mendelian ratios for number of genes model was unsuccessful. This was due to the complex segregation pattern for seed coat colour that was found between the observed and expected values from the cross. The F_2 populations showed varied colours from black to white with much difficulty in grouping them. This clearly shows the quantitative nature of inheritance of seed coat colour in cowpea as a result of multiple gene interaction. Egbadzor *et al.* (2014) noted that the study of seed coat colour as quantitative trait may be appropriate since colour pigmentation in plants may also be influenced by environmental factors such as solar radiation. The observations in this study indicate continuous variation in the seed coat colour rather than discrete classes. The identification of twelve (F_2) and thirteen (RF_2) colours in the segregation populations of the crosses means that several genes are involved in the seed coat colour inheritance in cowpea. Spillman and Sando (1980) suggested that, the inheritance of cowpea seed coat colour is controlled by five genes, while Fery (1980) cited by Mustapha (2009), also revealed that several of the genes governing the trait may be allelic.

Acquaah (2007) pointed out that, regarding the inheritance of seed coat colour of cowpea as qualitative trait will be misleading in breeding programmes, rather it should be regarded as quantitative traits because of many colours that are seen in the F_2 stage. The study conducted by Egbadzor *et al.* (2014) on six crosses of cowpea revealed that whiles individuals of two of the segregation populations could be grouped into definite seed coat colour patterns, it could not be done for the other four and this suggests that many genes

might be involved. This study also confirms that, several genes are involved in the inheritance of seed coat colour patterns in cowpea and they interact to produce varying patterns making the trait quantitative resembling what was noted in maize by Chandler *et al.* (1989). However, these findings contradict that of Asante (1999), which reported that two pair of genes are involved in the inheritance of seed coat colour pattern in cowpea. These contradictory findings imply the genetics of inheritance of seed coat colour in cowpea has not yet been understood due to the interactions and modifier genes that control the trait (Fery, 1980).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Based on the observations in the study conducted, the following conclusions were made;

1. High narrow sense heritability estimates (74%-99%) indicate that it is possible to transfer the genes that control early maturity to a late maturing variety in early generations. Heritability in the narrow sense is the result of the additive effect which is not broken in the next generation through the independent segregation of the alleles (Hugo *et al.*, 2014).
2. The degree of dominance for all the early maturity indicators examined varied from 0% -28% indicating predominance of additive gene effect in controlling these traits.
3. Both additive and non additive gene effects were significant in the expression of extra-early maturity in the cross. Additive x Additive (*aa*) and additive x dominance (*ad*) were the epistasis forms that were of great importance in the expression of the trait,

indicating that breeding procedures that make good use of these gene interactions can be employed to improve early maturity in cowpea.

4. Inheritance of extra-early maturity in cowpea is not influenced by maternal effects, suggesting that choice of maternal parent is not important in hybridization programme that aims at improving early maturity in cowpea.
5. Several genes are involved in the inheritance of seed coat colour patterns in cowpea and they interact to produce varying patterns making the trait quantitative resembling what was noted in maize by Chandler *et al.* (1989).

6.2 Recommendation

1. Pedigree, single seed decent and backcross breeding should be complemented with marker assisted selection to reduce long periods of time associated with these conventional methods and also improve on the accuracy of results.
2. Larger population constituting different seed coat colours of landraces and improved genotypes of cowpea need to be studied in order to determine precisely the mode of inheritance of seed coat colour pattern in cowpea and the number of genes governing the inheritance of this trait.

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APPENDICES

ANALYSIS OF VARIANCE

Appendix 1: ANOVA table for days to 50% flowering

Days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.629	0.314	0.07	
Block.*Units* stratum					
Treat	9	508.605	56.512	12.44	<.001
Residual	18	81.747	4.542		
Total	29	590.981			
LSD (5%):	3.4	CV(%):	5.0		

Appendix 2: ANOVA table for days to first flower initiations

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	12.867	6.433	1.53	
Block. *Units* stratum*Units* stratum					
Treat	9	374.300	41.589	9.88	<.001

Residual	18	75.800	4.211
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Total	29	462.967
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LSD(5%): 2.18

CV(%): 3.3

Appendix 3: ANOVA table for days to 90% pod maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	2.467	1.233	0.43	
Block.*Units* stratum					
Treat	9	1716.967	190.774	66.64	<.001
Residual	18	51.533	2.863		
Total	29	1770.967			

LSD(5%) 2.90 CV(%): 2.82

Appendix 4: ANOVA table for days to first pod maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
	2	10.562	5.281	1.88	
Block. *Units* stratum					
Treat	9	927.432	103.048	36.64	<.001
Residual	18	50.619	2.812		
Total	29	988.613			

LSD(5%): 2.87 CV(%) 3.43

Appendix 5: F₂ and RF₂ segregation seed coat colour pattern

No of seeds for RF ₂ segregation colours	Ratio	No of seeds for F ₂ segregation colours	ratio
1. 76	1	62	1
2. 132	2	70	1
3. 223	3	141	2
4. 360	5	180	3
5. 390	5	430	7
6. 750	10	456	7
7. 880	12	835	13
8. 987	13	957	15
9. 1605	21	2277	37
10. 2410	32	3010	49
11. 2430	32	3948	64
12. 3387	44	8035	130
13. 9097	119		
Total	293		329