



**MORPHOLOGICAL CHARACTERISATION OF LOW AND HIGH OIL
SUNFLOWER (*HELIANTHUS ANNUUS. L*) VARIETIES FOR USE IN MARKER
ASSISTED SELECTION.**

BY

ANTHONY CHINYUNDO

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LUSAKA**

SUPERVISOR: DR. C. GWANAMA

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DEDICATION

To my dearest Mother (late) and father, my sister Zefa and brothers Kamusisi and Cheelo. Above all the almighty God for letting it be so.

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ABSTRACT

Morphological characterization was done on three sunflower varieties; CCA81, Milika and Record in order to see morphological differences for possible use in marker assisted selection. The parameters that were looked at are leaf size, leaf shape, colour of leaves, number of leaves per plant, hairiness at top of stem, days to 50 % flowering and maturity, seed colour, presence of seed stripes, colour of seed stripes, position of seed stripes, shape of seed, weight of 100 seeds, kernel and oil percentages. Significant differences were noted in leaf size, plant height, days to 50 % flowering and maturity. Record had the highest oil percentage of 42.97, Milika 38.77 and CCA81 42.17. In the other parameters no significant differences were established. Variation among these characteristics is important because it allows for development of varieties adapted to specific environments or agro-climatic regions.

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1.0 INTRODUCTION

Sunflower, (*Helianthus annuus. L*), is one of the world's four major oil seed crops. The crop contributes close to half the world's requirement of edible oil, the other half being provided by cottonseed, rapeseed, soyabean and other minor crops. With the ever increasing demand for edible oil, the production of sunflower has greatly increased world over. For example between 1948 and 1952 Africa recorded a four fold (Carter, 1978). Of all the sunflower produced in the African continent, the annual statistics released by the Food and Agricultural Organisation (FAO) in the 1972-75 period show that most of increase in production occurred in South Africa. Although other countries in Africa are promoting the production of Sunflower, production is still at a small scale level (Carter, 1978).

In Zambia, Sunflower is an important oil seed crop that has the potential to save the country revenue on edible oil imports, estimated at 100,000 metric tones annually. The crop can also raise the living standards of the small scale farmer as an alternative source of income and edible oil. Sunflower is grown throughout Zambia, but more than 80% of the area planted is concentrated in the Eastern, Central and Southern province. Small scale producers cultivate approximately 95% of all Sunflower on plots of less than one hectare (Gillespie, 2000).

Sunflower can easily be produced by both small scale and commercial farmers to expand Zambia's exports since the crop is drought tolerant and can be profitably produced in marginal soils with reasonable yield. Among the major edible

oilseeds grown in Zambia, Sunflower is the easiest crop to produce at any level, small medium or large. However, its production has been quite erratic, since it was introduced in 1973. Poor marketing, policy changes, limited use and availability of inputs have contributed to the production fluctuations and ultimately to producers placing Sunflower and other oil seeds in a secondary role (Gillespie, 2000).

For efficient and economical production of Sunflower in Zambia, use of improved varieties by the farmers is necessary. Therefore, a morphological characterisation of three Sunflower varieties to be used in marker assisted selection was done.

Different traits can be assessed in young plants thereby reducing the number of years required for the normal breeding cycle. Selection efficiency using this method is increased because some traits are influenced by environmental factors and show a low response to selection based on phenotype (Diepenbrock, 1987). Marker assisted selection also accelerates breeding because RAPD markers are easy to score and interpret. DNA marker that are shown to be genetically linked to a trait of interest can be used for gene cloning and for trait introgression in plant and animal breeding programmes (Landergreen, et al 1988).

A morphological characterization of sunflower varieties was done in order to see morphological differences for possible use in marker assisted selection.

2.0 LITERATURE REVIEW

Sunflower, *Helianthus annuus* L is a member of the Compositae, a large and successful family of flowering plants occurring through out the world. It is distinguished from all other plants by its single stem and conspicuous, large inflorescence. Quantitative characteristics such as height, head size, achene size and time to mature vary greatly (Knowles, 1978). The cultivated Sunflower is a tall, erect, unbranched, coarse annual, with a distinctive large golden head, the seeds of which are often eaten.

2.1 Climate Requirements

Sunflower grows within a temperature range of 20-25°C, although a range of 8-34°C is tolerated without significant yield reduction. Temperature is known to affect seed and Oil characteristics, but its effects on plants growing in the field are often masked or modified by other environmental factors (Anderson, 1978). Sunflower is considered to be drought tolerant and often produces satisfactorily when other crops are seriously damaged (Weiss, 1983).

Sunflower grows well in soils ranging in texture from sandy to clayey soils. It grows well on neutral to moderately alkaline soils with a Ph range of 6.5-8.0 but dislikes acid conditions. Salinity affects plant growth and development, and some seed characteristic, usually oil content and also influences nutrient uptake (Robinson, 1978). Sunflower is considered an inefficient converter of plant nutrients as measured by its low harvest index (Weiss, 1983). Adequate nutrients are essential for high seed yield.

2.2 Morphology of Sunflower

2.2.1 Stem

The stem of cultivated Sunflower is typically unbranched although branched types frequently appear in commercial fields and are often used as male parents in producing hybrid cultivars. Stem dimensions and developments including branching are influenced strongly by the environment (Knowles, 1978). The stem is robust, circular in section, usually three to six centimetres in diameter but occasionally reaches ten centimeters and bears rough hairs and may have slight longitudinal ridges. It is usually green or greenish-yellow. The stem varies in height from one to three metres when full-grown (Weiss, 1983). Branches are usually small and occur towards the top of the stem and may vary in number.

2.2.2 Leaves

When the Sunflower seedling emerges from the soil, cotyledons unfold and the first pair of true leaves is visible at the tip of the shoot axis. The leaves vary in size, shape of the entire leaf, shape of tip and base, shape of the margin, shape of the surface, hairiness and petiolar characteristics (Knowles 1978). Leaves are usually alternate, occasionally opposite on the lower stem and alternate above, large, ovate and cordate.

The surface of leaves may be flat, concave or convex. The leaf surface may be scabrous (having short projections of the outer wall of epidermal cells that give a rapid texture), hispid (having short stiff hairs) or hirsute (having long silky hairs). Occasionally the leaf may be glabrous (Knowles, 1978). Leaves vary in intensity of green colour from light-green, green to dark green. On average 20 to 40 leaves are produced per plant depending on the variety (Weiss, 1983).

2.2.3 Inflorescence

The inflorescence of Sunflower is a head or capitulum, characteristic of the compositae family. It consists of 700-3,000 flowers in oilseed cultivars and occasionally up to 8,000 flowers in non-oilseed cultivars (Heiser, 1976). The head may vary from concave to convex. Head size may vary between cultivars, seasons, soil type, etc, and is commonly 10-30 cm in diameter but the greatest recorded has been 76 cm (Heiser, 1976).

The flowers are protandrous and normally cross fertilised and pollination is usually effected by insects. Little pollination is accomplished by wind (Putt, 1940). Ray flower petals of Sunflower are usually yellow but may vary from red to deep orange to very pale yellow or near white.

The ray flowers are normally asexual, but some may produce pollen. This disc flowers are perfect with petals and five anthers that are united in separate tubes. The disc flowers are arranged in concentric circles radiating from the centre of the head. The ray flowers open first and flowering then proceeds from the periphery to the centre of the head at the rate of one to four rows per day (Dedio and Putt, 1980).

2.2.4 Fruit

The fruit or achene of the Sunflower consists of a seed often called the Kernel. The seed varies in length from seven to 25 mm and four to 13 mm wide. The 100 seed weight of Sunflower varies from four to 20 g (Knowles, 1978).

The seed ranges in colour from black to white, but brown, striped or mottled seed can also occur. The colour of Sunflower seeds is determined by the presence or absence of pigment in each of the three different seed coat layers. Each layer may develop pigment independent of the outer layers (Putt, 1940). The outer layer or epidermis may be free of pigment, fully pigmented dark brown or black, or possess striped pattern of dark brown or black. White colour of the seeds results from a lack of pigment. Striping of the

seed is caused by uneven pigmentation in the epidermis (Putt, 1940).

However, the colour rating widely used in a selection programme is as follows: black, black with grey stripe, black with white/light-grey stripe, dark grey with white/light-grey stripe, grey with white stripe and white. (Weiss, 1983).

2.3 Chemical Composition of Seed

The seed constituents of Sunflower are normally a varietal characteristic, but there is a basic difference between seed produced under hot or temperature conditions (Goynes, et al, 1979). High temperatures during seed development can reduce total oil content. The oil percentage of whole Sunflower seeds depends on both the percentage of hull and percentage of oil in the kernel. The kernel to husk ratio is of prime importance for profitable oil production and varies considerably with larger seeds generally having the lowest kernel percentage. Black and striped usually have a lower kernel to husk ratio and total oil content than white seeds, and there is a negative relationship between oil content and hull thickness (Anand and Chandra, 1979).

Sunflower oil is comprised primarily of palmitic, stearic, oleic and linoleic acids accounting for about 90% of the total (Cummins, et al 1967). Sunflower oil also has a very low content of linolenic acid. There is an

inverse relationship between Oleic and Linoleic acid which is highly influenced by the environment especially temperature during the growing season (Canvin, 1965). The average composition of oil from temperate crop is 55-60 percent linoleic acid and 25-30 percent oleic acid but seed produced under high temperature may contain 65 percent oleic and 20 percent linoleic acid (Weiss, 1966).

2.4 Uses of Sunflower

The oil extracted is of high quality with over 90% of unsaturated fatty acids. Sunflower oil is also classified as a semi-dried oil and therefore used for making paints, varnishes, soap, detergents as well as plastic materials. The crop can also be used as livestock feed.

Sunflower cake and concentrates, as by-products, have considerable nutritional potential because they lack toxic factors and they are a source of calcium, phosphorous and nicotinic acid (Carter, 1978). One of the sunflower seed crushing by-products which is the hull material can be ground and used as a filler or roughage for livestock rations. The seeds are fed to birds; for example whole kernels are fed to parrots.

3.0 METHODS AND MATERIALS

The experiment was conducted on a farm in Kafue during the 2001/2 growing season.

The land was ploughed and thereafter harrowed so as to produce a fine tilth. The three *Sunflower varieties CCA81, Milika and Record*, were planted in a single block with each plot containing twenty plants. The spacing within the single plots was 20 cm while that between the three plots was 100 cm. Border rows were also planted on each side of the plots. The planting was done on 7th February 2002.

Management of the field was done by ensuring that the field was free of weeds. Normal fertilizer rates of compound X fertilizers. were applied. Irrigation of the field was performed twice a week.

3.1 Data Collection

At maturity, Morphological characterisation of the three varieties on the following parameters was carried out; Leaf size, leaf shape, colour of leaves, number of leaves per plant, hairiness at top of stem, days to 50% flowering, days to 50% flowering, seed colour, presence of seed stripes, colour of seed stripes, position of seed stripes, shape of seed, weight of 100 seeds, kernel percentage and oil percentage

4.0 RESULTS AND DISCUSSION

4.1 Leaf Parameters

The mean leaf sizes were 15.4 ± 0.79 cm for CCA81, 10.3 ± 0.60 cm for milika and 14.8 ± 0.42 cm for Record. Leaf size was not significantly different because CCA81 and Record but different between these two and Milika. Milika has the smallest leaf size while CCA81 and Record had medium sized leaves.

Milika and Record had leaf shapes that were predominantly cordate while that of CCA 81 was triangular.

The predominant leaf colour for all the three varieties was medium green. Leaf shape and colour determinations were done subjectively as they are qualitative traits.

No significant differences were noted in the results for number of leaves between CCA 81 and Milika, CCA 81 and Record, Record and Milika. The highest mean number of leaves was recorded in the variety Record which had 23 followed by Milika with 20 (Table 1). CCA 81 had the least number of leaves at 19. Within a variety, the final leaf number and leaf size per plant is mainly an effect of environment, i.e, plant population, soil moisture and temperature. The number of leaves on a plant has been

shown to be related to seed yield and seed oil content (Weiss, 1983). It was shown that a 50 % defoliation of the leaf area, which depends on both leaf size and number of leaves, resulted in a significant depression in seed yield.

The most critical period for defoliation to occur is between bud formation and flowering (Weiss, 1966). Heiser (1976) has reported that the five and ten lowest leaves on the stem contribute only one and ten percent, respectively, to the final seed yield and that the lower leaves may contribute directly to oil synthesis in developing seed. Photosynthesis by leaves is related to the age of the leaf and the stem position and is greatest in the highest leaves (Weiss, 1983). There also appears to be some association between leaf number and time to maturity, for plants with numerous leaves are usually late in maturing (Knowles, 1978).

A variety with a high number of leaves as in Record is advantageous as it will have high seed yield and oil content and is therefore desirable. Plants with fewer number of leaves will mature early as in CCA 81.

The mean plant heights were 1.24 ± 0.11 m for CCA81, 1.32 ± 0.11 m for Milika and 1.59 ± 0.65 m for Record. Plant height was significantly different between CCA81 and Record and between Milika. It was not significantly different between Milika and CCA81.

Plant height is an important trait in sunflower. This is because plant height influences lodging. Varieties that have tall plants are susceptible to damage by wind.. Lodging in tall varieties can therefore cause a lot of losses in terms of yield. Short varieties should therefore be used whenever possible, even if the oil content may be lower than in taller local varieties. Shorter varieties have the additional advantage of allowing the soil to dry out more quickly before harvest since there is less cover (Weiss, 1983).

Record being the tallest among the three varieties can be easily susceptible to lodging while CCA 81 with the least height is the best variety as far as lodging is concerned. A cross can therefore be made between Record and CCA 81 so as to produce a plant that will resist lodging as well as have an adequate number of leaves that will make it have high seed yields. Taller varieties of sunflower have the advantage of being drought resistant as compared to shorter varieties (Knowles, 1978). However, a short, sturdy, single – stemmed plant with leaves mainly at the top of the stem is a most desirable type for mechanized production. Genetic control of lodging probably is complex because it depends on several characters including plant height, stem strength and diameter, and the type of root system (Fick, 1978).

CCA 81 and Milika were found to be low haired while Record had medium hair on the stem. The presence of hairs on the stem depends on the genotype of a particular variety under consideration (Knowles, 1978).

4.2 Days to 50% Flowering

CCA 81 was the earliest in flowering among the three varieties to attain 50% flowering with 64 days followed by Milika with 75 days and the last was Record with 81 days.

Flowering uniformity was observed in Milika which has over 17 plants at 50 % flowering, followed by Record with 13 plants and the least was CCA 81 with 11 plants.

Sunflower seed is considered mature when the back of the head turns yellow, although the seed will contain 20 to 35 % moisture at this stage of plant development (Weiss, 1983). CCA 81 attained 50 % maturity earlier than the other varieties with about 103 days, followed by Milika with 109 days. Record had the longest number of days to reach 50% maturity with about 113 days. Days to maturity often are closely correlated with days to flowering, although genetic differences in the time required from flowering to maturity exist (Heiser, 1976).

It has been observed from the results that the 'tall' variety had the longest number of days both in 50% flowering and maturity while the shortest variety, CCA 81 had the shortest number of days to 50% flowering and maturity, while Milika had the greatest uniformity. Evenness of growth, ripening and moderate height are usually essential requirements for successful mechanical harvesting, particularly combine harvesting.

It is therefore essential to plant a variety that has uniformity in its growth patterns such as Milika. This is because varieties that do not exhibit uniformity, ripen over a prolonged period or grow unevenly with varying head size, all of which make mechanical harvesting extremely difficult and costly. Leaving mature plants standing in the field because many others are unripe also increases bird damage which is a major cause of seed loss in many temperate and tropical countries (Weiss, 1983).

In order to obtain plants that will mature early and also have uniformity in both maturity and flowering, a cross can be made between CCA 81 and Milika. The importance of early maturity and uniformity cannot be overemphasized. Since this was a preliminary characterization and in the absence of replication, no t-test could be done.

Delayed harvesting due to non-uniformity in growth of plants also allows unwelcome changes in seed oil composition such as an increase in free fatty acids (Weiss, 1983).

4.3 Seed Parameters

The predominant seed colours in Milika and Record were black while that CCA 81 was grey. The colour of sunflower seeds is determined by the presence or absence of a pigment in each of the three different seed coat layers. Milika and Record were therefore found to be fully pigmented with a black colour while CCA 81 was found not to be fully pigmented. The inheritance of seed colour has been considered to be complex because of the numerous combinations of pigment that may occur in different layers and that several genes that control these combinations have been identified (Fick, 1978).

Many growers equate dark seed with the oil market and light or striped seed with birdseed or confectionary sectors. Although there is often a relationship between a dark hull and high average oil-seed content an increasing number of hybrids have light coloured seed with light oil content. This was evidenced in the results obtained for the oil content. Record that had a dark or black colour had an oil percentage of 42.97 while the light coloured seed (CCA 81) also had a high oil percentage of 42.17. Milika had the lowest oil percentage of 38.77.

Oil percentage of whole sun flower seeds depends on both the percentage of hull and the percentage of oil in the kernel. Oil percentage is also generally considered to be quantitatively inherited. Information on the rate of development of seed characterisation, especially in respect of oil formation is important as it affects many agricultural operation such as irrigation, defoliation, harvesting data etc. In respect of the rate of seed oil formation, there appears to be little difference between low and high oil content varieties, but that total seed oil content is related to rate of cotyledon cell formation. This is faster in high-oil content seed than in low oil content seed (Weiss, 1983). Fertilizers may also affect seed characteristics, e.g, phosphate can increase seed oil content and nitrogen reduce it and therefore an optimum level of both phosphate and nitrogen should be applied in order to achieve highest oil content.

Seed stripes were present in all the three varieties. CCA 81 and Milika possessed white stripe while Record had grey stripes. Seed striping is caused by the uneven pigmentation in the epidermis.

The position of these stripes were marginal in Milika and CCA 81 while in Record, these stripes were observed on both lateral and marginal sides of the seeds (Table 2).

CCA 81 and Milika's seed shape were ovoid wide while that of Record was rounded.

The weight of sunflower seeds is greatly effected by both additive and non-additive gene effects and the type of gene action controlling seed weight depends on the genotypes being evaluated (Fick 1978). The 100 seed weight for Record was found to be 7.2 g followed by CCA 81 with 6.7 g. The lowest 100 seed weight was observed in Milika which had a weight of 6.5 g. A statistical test could not be performed on the above results as this was a preliminary characterization with no replication.

The seed weight on its own cannot give a good indication of the marketable yield unless the kernel percentage is determined. This is because there usually are unfilled seeds at the head centres. The kernel percentage is of prime importance for profitable oil production and varies considerably with larger seeds having the lowest kernel percentage. Record had the highest kernel percentage of 68 % and the least was noted in CCA 81 with 60 %. Milika had an intermediate kernel percentage of 63 %. The correct time of planting not only affects yield but also the kernel percentage.

Record with the highest Kernel percentage of 68 % also had the highest oil content of 42.97 %. It would therefore be desirable to make a cross

between CCA 81 and Record so as to produce plants that will have a low kernel percentage but still maintaining a high oil content. This would be advantageous in that hulling problems in sunflower may be reduced. This is because the difficulty of separating the hulls and the Kernels presently limits production of flours and protein isolation. Therefore selection of seed varieties whose seed has a space between the hull and kernel without *loss of oil content would materially assist seed processors, and increase the commercial attractiveness of sunflower seed in the crushing industry.* Record is the only variety that seems to meet the above requirements although it needs to be crossed with CCA 81 as earlier mentioned.

Morphological characterisation is therefore important as it outlines genetic variation among characters associated with plant growth and resultant morphological or physiological differences. These serve as the basis for development of lines and varieties with improved agronomic traits. Variation among characters such as plant height, flowering and maturity are particularly useful because it allows for development of varieties adapted to specific environments or agro-climatic regions.

Table 1: Morphological characteristics of three sunflower varieties for use in marker assisted selection.

TRAITS									
VARIETY	Leaf Size	Leaf Shape	Colour of Leaves	Number of Leaves	Plant Height (m)	Hairiness at top of stem	Days to 50% Flowering	Days to 50% Maturity	
CCA81	15.4 ± 0.79 a	Triangular	Medium Green	19 ± 2.2 a	1.24 ± 0.11 a	Low Haired	64	103	
MILIKA	10.3 ± 0.60 b	Cordate	Medium Green	20 ± 2.1 a	1.32 ± 0.11 a	Low Haired	75	109	
RECORD	14.8 ± 0.42 a	Cordate	Medium Green	23 ± 2.2 a	1.59 ± 0.65 b	Medium Haired	81	113	

Means followed by the same letter are not significantly different at p = 0.05

Table 2: Seed characteristics of three sunflower varieties for use in marker assisted selection

VARIETY	TRAITS							
	Seed Colour	Presence of Seed stripes	Colour of Seed Stripes	Position of Seed Stripes	Shape of Seed	Weight of 100 Seeds (g)	% Oil	Kernel %
CCA81	Grey	Present	White	Marginal	Ovoid wide	6.7	42.17	60
MILIKA	Black	Present	White	Marginal	Ovoid wide	6.5	38.77	63
RECORD	Black	Present	Grey	Lateral and Marginal	Rounded	7.2	42.97	68

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6.0 APPENDIX

PAIRED SAMPLES TEST

Parameter	Mean	Std. deviation	Std Error mean	Sig (2-tailed)
Plant height for Milika vs Record	-27.2000 ^a	20.5307	6.4924	0.002
Plant height for CCA81 vs Milika	-7.4000 ^b	35.3465	11.1776	0.525
Plant height for CCA81 vs Record	-34.600 ^a	34.0301	10.7612	0.011
Number of leaves for CCA81 vs Milika	-1.1000 ^b	7.0624	2.2333	0.634
Number of leaves for CCA81 vs Record	-4.2000 ^b	8.2973	2.6238	0.144
Number of leaves for Milika vs Record	-3.1000 ^b	6.9514	2.1982	0.192
Leaf size of Milika vs Record	-2.000 ^a	1.3333	0.4216	0.001
Leaf size of CCA81 vs Record	-1.4000 ^b	2.5033	0.7916	0.111
Leaf size of CCA81 vs Milika	-0.6000 ^b	1.8974	0.6000	0.343

Note: a means that there a significant difference between the parameters at 95% confidence interval of the difference b means that there is no significance difference between the parameters at 95% confidence interval of the difference.