DEVELOPMENT OF DROUGHT TOLERANT WHEAT LINES THROUGH CHROMOSOME ELIMATION TECHNIQUE

BY

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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other University.

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the second most important staple cereal in Kenya after maize. Its production has been concentrated in the cool high altitude areas ranging between 1800 – 3000m above sea level. There is lack of varieties suitable for the dry regions which make over 83% of the total Kenya landmass. This study was aimed at developing drought tolerant varieties of wheat suitable for growing in the semi-arid regions through doubled haploid (DH) breeding technique. This was accomplished through the following steps: (i) F_1 crosses were produced by crossing three drought tolerant varieties namely Kenya Mbweha, Duma and Ngamia with two high yielding commercial varieties namely Kenya Chiriku and Kwale, (ii) the haploids were produced through chromosome elimination by crossing the F_1s with maize pollen, and (iii) the doubled haploids (DH) were produced by treating the haploids with colchicine. Twenty DH lines were produced from this process and tested for drought tolerance and yield characteristics at the National Plant Breeding Research Centre (NPBC) Njoro under a rain-out shelter. Of the twenty DH lines four ($DH_1$14, $DH_1$2, $DH_1$7 and $DH_1$12) were able to combine drought tolerance and yield and performed better than the higher parents especially on grain count per head and 1000 kernel weight indicating heterosis. Most of the other DHs had their grain weight and number per head between the two parents. There was wide variation between the different DH lines indicating high potential for selection for various agronomic characteristics. Heterosis was realized on a number of characteristics in the DH lines. Through this technique the period of developing pure lines was reduced to two years as compared to seven years taken through conventional breeding methods.

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CHAPTER 1

1.0 INTRODUCTION

1.1 Wheat production in Kenya

Wheat (*Triticum aestivum* L.) is the second most important cereal crop after rice in the world and ranks second to maize in consumption in Kenya. It has been grown since the early historic times as is evidenced in the archeological finding and expedition by plant investigators (FAO 1970, 1986; Bajaj 1991).

In Kenya wheat has been grown from the turn of the 20th century at first by large-scale farmers and later by small-scale producers (Kinyua, 1997). It was traditionally cultivated in the high attitudes ranging between 1,800m and 3,000m above sea level. The main wheat producing districts in Kenya include Nakuru, Narok, Uasin Gishu, Trans-Nzoia and Nyandarua (Acland, 1980; Kamidi, 1995 and Kinyua, 1997). Small-scale production is found in Eastern Kenya mainly Meru and Laikipia districts (Jaetzold and Schmidt, 1983). Recently wheat has been introduced into the lower dryland areas of Machakos, Naivasha, Koibatek and Lower Narok, among others (*Kinyua*, 1997).

Wheat production in Kenya showed a slow growth rate of 0.9% while consumption of its products spurred by population growth, urbanization and changing consumption habits, has been increasing by 5.1 percent per year (FAO, 1994). The increased demands have been met through imports. Between 1972 and 1991, for example, wheat import increased at a rate of 13.1% per year. Over a period of twenty years (1980-1999) Kenya has gone from being self sufficient in wheat production to being net importer to meet its consumption needs (Kamidi, 1995; FAO, 1999). By 1995 Kenya was importing 50% of its wheat requirements (Kamidi,1995; Kinyua, 1997).

This trend can be reversed by increasing productivity in existing wheat areas and developing suitable technologies for potential expansion in dryland areas by developing high yielding drought tolerant and stable varieties, better crop management package, appropriate plant protection methods and improved research-farmer-extension linkages for enhanced technology dissemination (Kamidi, 1995; KARI, 1995).

Wheat is grown only in 2% of the high altitude areas which make about 17-18% of the country's land area (Kinyua *et al.*, 1989; Kinyua, 1997). It has to compete with other agricultural enterprises, such as maize, tea, coffee and dairy farming. Meanwhile the marginal areas with annual rainfall from 650-850mm remain agriculturally underutilized and can form very valuable land under which suitable varieties of wheat can be grown. Development of wheat varieties suitable to marginal areas would make a great impact in wheat production (Nyachiro, 1991). The introduction of wheat in the non-traditional areas has serious economic implications such as high cost of production and low yield (Byerlee and Longmire, 1986). About a third of the areas planted with wheat in these non-traditional areas suffer from drought at one stage or another (Kohli *et al.*, 1990).

Drought tolerance is a complex phenomenon in both its physiology and its genetics. It is usually estimated by yield potentia.Yield is determined by a multitude of physiological, biochemical and metabolic processes whose genetics are largely unknown (Blum, 1998). Also the various environments under which genotypes are tested and under which their stability is measured are largely undefined in the biological sense.

Wheat improvement by traditional methods has been practiced since the beginning of the 20th century however, it has now been realized that these methods are insufficient to make any further breakthrough or to cope with the increasing demand. Although numerous varieties are

released every year around the world, they do not last long, and long term objectives cannot be realized unless more genetic variability is generated (Bajaj, 1990). Since the success of a crop improvement program depends on the extent of genetic variability in the base population, innovative approaches have to be incorporated in order to develop novel high-yielding disease resistant and drought tolerant cultivars. This is possible if conventional breeding methods are combined with *in-vitro* biotechnology and genetic engineering techniques among others. Plant biotechnology has come of an age where it offers the most potent emerging techniques for the induction of genetic variability and has far reaching applications.

1.2 The Doubled Haploid (DH) Technique

Due to heterozygosity and heterogeneity of the inbreeding generations and the heterogeneity of the drought areas over location and years it makes it difficult for conventional breeding methods to be effective in selection for drought tolerant wheat lines. DH lines are homozygous and heterogeneous (Baenziger, 1996), and superior lines can be selected much more easily.

In DH technique two methods of producing large number of haploids are known:
(i) Chromosome elimination following wide hybridization, and (ii) *in-vitro* culture of male gametes. Chromosome elimination has been established as a method of producing haploids in wheat either from crosses with barley (*Hordeum bulbosum*) or maize (*Zea mays*) pollen (Laurie and Bennett, 1988). The use of maize pollen has become more popular in commercial DH breeding due to the fact that there is no strong wheat genotypic difference and also the frequencies of haploids are high enough (Pickering, 1992; Laurie and Bennett, 1988; Kasha, 1995).

The chromosome elimination technique has advantages over anther culture in a number of ways: (i) nearly all embryos produced are haploid and have little spontaneous doubling of chromosomes, (ii) there is no occurrence of albinos, and (iii) a random sample of gametes is obtained (Kasha 1995; 1997).

The autoploid haploid is used to simplify the genetics of traits and for reconstructing new combinations of genes at the hexaploid level. The overall feature of haploid use is to save time (generations) in the breeding programs. Selection based on comparison of the uniform doubled haploid progeny lines is much easier than segregating progeny rows. The DH lines also are a random selection of gametes (single gametes per DH line) and require fewer lines to find the superior gametes than by using single seed descent (SSD) (two gametes per line) (Kasha, 1995).

Picard *et al.*, (1990) reported that the DH method is much more efficient compared with other three conventional methods, i.e. pedigree, bulk and SSD, especially when dealing with quantitative characters. The doubled haploid technique shortens the long process in conventional methods and the homozygous lines developed soon after hybridization allow better discrimination between genotypes, within crosses, better discrimination between crosses and greater selection response across generations (Snape *et al.* 1988; 1982). The DH method is more applicable in maintaining the genes of interest in selecting for complex characters like drought tolerance and yield because none of the genetic combinations are lost as in natural selection.this because allelic interactions are absent and thus the phenotype would be a direct reflection of the genotype.

1.3 Objectives

The general objective of this project was to develop wheat varieties which are drought tolerant, and high yielding through doubled haploid(DH) technique and hence increase wheat production in Kenya.

The study addressed the following specific objectives:

(i) to develop F_1 hybrids between three drought tolerant lines namely Duma, Ngamia and Mbweha and two high yielding but drought susceptible commercial varieties Kwale and Kenya Chiriku.

(ii) to develop haploids from the F_1s by crossing F_1s with maize

(iii) to develop Doubled Haploid (DH) lines from the haploids obtained through colchicine treatment, and

(iv) to test the doubled haploids for recombination of drought tolerance and high yield under simulated drought conditions.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Drought tolerance in wheat

Drought is a multidimensional stress affecting plants at various levels of their organisation (Blum, 1996). The effect and plant response to drought at the whole plant level is most complex because it reflects the integration of stress effects and responses at all underlying levels of organisation over space and time (Blum, 1996).

What makes a particular drought site/cycle often so little representative of an entire drought target environment is the spatial and temporal variability within droughty environments. The potential diversity of environmental conditions is larger at lower yield levels than at the higher yield levels, (Arntzen, 1986). There are more different ways of obtaining low yields than there are for achieving very high yields. Thus any increase in genotype by environment interaction at lower yield levels as a result of the inherent increased environmental diversity will make selection at any one stress site in any one year less effective in regard to its relevance to the entire stress target environment (Maarten, 1994).

The Heterogeneity of the target environment makes it difficult to determine how exactly production is most affected by drought. Maarten (1994) defines two philosophies regarding which environment should best be used for selection: (i) optimal conditions with water and nutrients, non-limiting, guarantee the best selection efficiency, since yield potential is maximized and hence heritability and subsequent genetic gain from selection. Evaluation under drought must follow, (ii) drought conditions represent the target environment and therefore provide the relevant selection environment. The most urgent problem in breeding for

drought tolerance in wheat is the construction of a multiple selection index that takes account of the target environment, the physiological issues involved, and availability of methodology and the economic constraints of the program.

The improvement of yield under stress must combine a reasonably high yield potential with specific plant factors which would buffer yield against a severe reduction under stress (Blum, 1983). On the other hand, potentially lower yielding genotypes occasionally have been found to perform very well under severe drought stress conditions (Blum, 1982; Ceccarelli, 1987). Biswanger and Barah (1980) divided the relevant plant independent variables into three types, control variables, site variables and weather variables. Weather variables include such factors as total rainfall, its distribution, soil moisture, sunshine hours, cloudiness, temperature etc. They vary across locations as well as over years and do so in a similar manner. In the case of drought, we are dealing with "weather variables". The similarity allow us to use (spatial) adaptation as a measure of temporal stability in dry areas, while this would for example, not be the case to response to soil acidity. Hence, multilocational testing, a procedure to gauge spatial adaptation is used extensively by the bread wheat program to identify temporally stable drought tolerant germplasm (Maarten, 1994).

Since shortage of water is a chief cause of variation in wheat yields, especially in the dry areas, it is needful to predict the likely effects of variation in rainfall. The genotypic differences under drought can only be studied under the right conditions. However, a rain-out shelter could be a good alternative (Kinyua, 1999). Mobile rain-out shelters exclude rainfall while allowing other environmental variables to fluctuate naturally (Coles *et al*; 1991). Under these shelters the water availability is under the control of the experimenter (Kinyua, 1999.)

2.2 Doubled haploid (DH) technique

Scientific approaches to wheat breeding have their history in the development of the pedigree system after the rediscovery of Mendel's laws at the beginning of 20th century. There has been a consistent search for technologies that can convert plant breeding from an art to a science. A search stimulated by need to increase food production, the necessity of counteracting the evolution of pests and diseases, introduction of changes in agronomic practices, environmental perturbations, the commercial aspiration of competitions (Snape, 1998) and for the developing countries food self sufficiency (Fisher, 1990).

Practical breeding programs of self-pollinated crops such as bread wheat must include a process of genetic fixation for uniformity of agronomic traits after genetic recombination to increase variation. Repeated selection of heterozygous materials following hybridization can increase uniformity but many generation cycles are required to reach homozygosity especially in loci associated with agronomic traits (Inagaki, 1996). It requires many generations of self pollination and selection to achieve the levels of homozygosity and hence genotypic stability required for a variety to be released (Snape, 1998). This makes the three conventional breeding methods namely bulk, pedigree and single seed descent (SSD) less effective in selection for drought tolerance.

One of the major contributions of tissue culture techniques to wheat breeding has been the development of doubled haploid (DH) system which can short circuit this process (Snape, 1998). Doubled haploids derived from heterozygous materials show complete uniformity when used as recombinant inbred lines in selection procedure (Inagaki, 1996). The DH lines

reduce the selection period from 13 to 5 years (Inagaki, 1996). The lines also are stable over environments (Choo *et al.*, 1985). Ba Bong and Swaminanthan (1995) indicated that for traits expressing predominately additive genetic effects the derived DH lines reach the heterosis level of the F_1 hybrid.

An added and significant advantage is that these systems not only speed up the advance to homozygosity, but increase selection efficiency (Snape, 1989). This is obtained because of the greater proportion of additive genetic variation available to selection for quantitative traits and absence of dominance effects for major genes (Snape, 1988). This allows better discrimination between genotypes within crosses, better discrimination between crosses, and greater selection response across generations (Snape , 1982,1998).

The discovery by Laurie and Bennett (1988) that chromosome elimination in wheat could be exploited by using the intergeneric cross with maize pollen has led to a commercially exploitable genotype independent system. This system is now enabling large population of homozygous recombinant lines to be produced at reasonable cost.

Crossing techniques have been improved to allow large scale emasculation and high fertilization frequencies. There have been improvements in the past pollination's application of plant hormones which allow high frequency of embryo survival and good quality embryos with a high frequency of germination to be formed. Improved media have been developed to increase the development of healthy haploid seedlings. Finally, chromosome doubling technique have been refined to ensure high survival yet efficient doubling (Snape, 1998).

2.2.1 Production of haploids

Wheat haploids are polyhaploids (n=3x=21) because wheat plants have three genomes A, B and D each with seven homologous chromosomes (Inagaki, 1990). There has been tremendous progress in haploid production since the first wheat pollen plants were produced simultaneously in China by Ouyoung *et al* (1973) and in France by Picard and de Buyser (1973).

The theoretical and practical usefulness of haploids has been recognized since the 1920's when spontaneous haploids were discovered (Kudirka *et al.*, 1986). There are several practical drawbacks in recovering of homozygous diploid plants from spontaneous haploids. First spontaneous haploids occur at very low frequencies and second, chromosome doubling which is necessary if these plants are to set seeds is not always successful (Kudirka *et al.*,1986). Haploid plants can be produced by a number of methods and have been reported in well over 200 plant species (Kasha *et al.*, 1995). Two major methods for producing wheat haploids are: (i) using microspores (pollen), and (ii) using megaspores (egg cells). These have been examined over the last two decades (Inagaki, 1996). The method for producing haploids from cultured pollen has developed as an anther or pollen-culture technique, and is still constrained by the difference in the responses of wheat genotypes (Kisana, 1993; Zhou, 1996). Since the early 70's other techniques have been developed in order to produce haploid wheat plants; the bulbosum technique used for some particular genotypes (Barclay, 1975), the Salmon method (Kobayashi and Tsunewaki 1978; Tsunewaki *et al.*,1984) and the wheat x maize crosses (Laurie and Bennett 1988). Limited numbers of haploids can also be produced using irradiated pollen (Natarajan and Swaminathan 1958; Snape *et al.*, 1983) and unpollinated ovary culture (Zhu Wu 1979; Yan *et al.*,1979).

Despite the intensive efforts to increase anther response (Henry and de Buyser, 1990), its use has remained marginal in wheat breeding programs. The major limitations to a broad exploitation of anther culture has been its genotypic dependency (Lazar *et al.*, 1984, Feroughi-Weter and Zeller 1990). Wide crosses followed by elimination of the genome of one parent have been an alternative method for inducing haploid zygotic embryos and subsequent plants (Lafebure and Devaux, 1996; Snape, 1998).

2.2.2 Methods of haploid production in wheat x maize crosses

Kasha and Kao (1970) reported the production of haploids in barley following the hybridization of barley (*Hordeum vulgare*) with *H. bulbosum*. Haploids were regenerated by rescuing the embryos in a nutrient medium. Chromosome elimination was proposed on the basis of the influence of the genome balance in reciprocal crosses between the diploid and autotetraploid levels of these species. It was later confirmed by cytological (Wo and Kasha, 1975; Laurie, 1988) and genetical studies (Pickering and Devaux, 1992).

Chromosome elimination in other interspecific crosses between wheat and *H. bulbosum* was subsequently formed (Pickering, 1992). Whereas haploids could be produced in wheat by this system, the frequencies in most genotypes of wheat were below 1% and could not be economical for breeding purposes (Kasha *et al.*, 1995). It was found that using maize (*Zea mays)* pollen on wheat there were no strong wheat genotypic differences (Laurie and Bennet, 1988). Other plants of the Graminea Family namely pearl millet and sorghum have been successfully used for chromosome elimination (Inagaki, 1995).

The chromosome elimination method involves pollination of the bread wheat with maize and subsequent elimination of the maize genome (Comeau *et al.*,1988; Laurie and Bunnett, 1989;

Suenaga and Nakajima ,1989; Riera-Lizaraza and Mujeeb-Kazi, 1990). Although similar to the *H. bulbosum* method this method has added advantage of being genotype independent as it is largely unaffected by the presence of dominant alleles at the Kr loci which restrict crossability with *H. bulbosum* (Laurie and Bennet 1987).

Intergeneric crosses of hexaploid wheat with maize result in the formation of globular embryos following preferential elimination even when wheat varieties not crossable with *Hordeum bulbosum* L are utilized in crosses (Zenktelen and Nitzche, 1984; Laurie and Bennet, 1986, 1987).

Effect of parental genotypes on haploid production through wheat x maize crosses is still not clear issue in the literature. Suenaga and Nakajima (1989) observed a significant influence of the maize genotype on the success of haploid plants produced via chromosome elimination in wheat x maize crosses. Inagaki & Tahir (1990) on the other hand, found that significant differences in crossability only depend on the wheat genotype. Laurie and Reymondie (1991) crossed commercial wheat lines with one maize F_1 hybrid, and found a difference in haploid production only between winter and spring type wheats but not within the groups.

It appears that for haploid wheat production specific maize genotypes are more suitable than others. From a practical point of view a convenient maize cultivar which is a good pollinator, easy to cultivate in a green house, grows quickly, produces a large amount of pollen and does not grow too high for ease of pollen collection, should be used (Bitch *et al.*, 1998).

The embryology of wheat x maize crosses was studied by Laurie and Bennett (1986, 1987, 1988, 1989 and 1990) on whole mount preparation stained with the Feulgen method and acetic carmine. Wang *et al* (1991) and Sun *et al.,* (1992) analyzed the cross on paraffin-

sectioned material. Laurie and Bennett (1986) found that the embryo were initially hybrids between wheat and maize, but the embryo lost the maize chromosomes during the first cell division and became haploid embryos of wheat (Laurie and Bennett, 1988m 1989; Laurie and Reymondie, 1991). Unlike *Hordeum vugare* x *H. bulbosum* crosses where the parental ploidy level has a marked effect on the stability of the progeny karyotype (Kasha and Kao, 1970; Lange, 1971), in the wheat x maize crosses the pollinator genome was rapidly and completely eliminated, even when the genome relations changed using tetraploid maize (Matzk and Mahn, 1994).

The pollen tubes germinate about 30 minutes after pollination, the tubes (two per stylodia) depending on the environmental factors reach the micropyle 5 days after pollination. With 2,4-D treatment the number of pollen tubes that reach the micropyle are more and take three days. The maize pollen tubes follow the way usual for wheat pollen tubes described in detail by Vishnyakora and Willemse (1994). This shows that maize pollen tubes are sensitive to the attraction signals leading them to the wheat micropyle (Wedzony and Van Lammeren, 1996). Such attractions are also likely to exist in other related crosses e.g. wheat x pearl millet (Ahmad and Cameau, 1990) wheat x sorghum (Ohkama *et al.,* 1992), oat x maize (Rini and Dohleen, 1990), and barley x maize (Furusho *et al.,* 1991). In the intergeneric crosses of cereals the growth of the alien pollen tubes is retarded and aberrant but not inhibited and still depends on the same pathway information as in selfed combination (Wedzony and Van Lammeren, 1996).

The programic phase in wheat x maize crosses lasts several hours up to one day after pollination but 2,4-D treatment significantly increases pollen tube arrival at the micropyle and the rate of successful egg cell and/or control cell fertilization (Wedzony and Van

Lammeren,1996). Fertilization of bread and durum wheat by maize is possible but plant recovery can be difficult as young seeds left on the plant rapidly abort (Laurie and Bennet, 1988a; O'Donoghue and Bennett, 1994). Three methods have been used to grow and develop haploid seedlings from bread wheat x maize crosses. Laurie and Bennett (1988a) planted whole spikelets 2 days after pollination on MS medium (Murashige and Skoog 1962) supplemented with sucrose and the synthetic auxin 2,4-D (Mathias and Boyel, 1988) followed by embryo culture 3 weeks later. Comeau *et al* (1988) recovered plants using ovule culture and a medium high in sugars with subsequent transfer to modified Norstag-II Medium (Norstag 1973). The third method involves treatment of ovaries *in-vivo* with a solution of 2,4-D until the embryo became big enough to be cultured and germinated on standard embryo culture medium. The third method has higher embryos rescue and has been adopted by most of the breeders (Inagaki, 1996).

The 2, 4-D application has been successful through direct placement inside the floret (Laurie *et al.,* 1990), spraying (Riera-Lizarazu and Mujeeb-Kazi, 1990), injection into the upper most internode of the wheat stem (Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990; Riera-Lizarazu and Mujeeb-kazi, 1990) and by placement of detached spikes into an aqueous solution (Riera-Lixarazu and Mujeeb-Kazi, 1990).

When *Triticum* species are pollinated with maize the ovaries remain small and gradually dry out on the plant whether they are fertilized or not (Laurie and Bennett 1988a, O'Donoughue 1990). The Maize pollen tubes are unable to provide the hormonal stimulus required for fruit development (Comeau *et al.,* 1988) and 2, 4-D application compensates partly for this. The effect of 2,4-D on ovary enlargement is correlated with its concentration (Marshalls *et al.,* 1983).

The time of auxin application after pollination was found to be a very important factor in promoting embryo development by Matzk and Mahn (1994). An increase in time between pollination and auxin application resulted in higher percentage of embryo formation. Auxins should not be applied earlier than 20 hours after pollination. Therefore they should be applied at least day after pollination. No significant variation influence on induced grains or embryos was found between the application methods tested (spraying, dropping and dipping) (Matzk and Mahn, 1994). A single treatment with an aqueous solution of 50-100ppm dicamba or 2,4-D, the most efficient synthetic auxins is sufficient for stimulation of embryo development. Auxin penetration and hydration of the embryo sacs occur shortly after treatment and fertilization of such ovules are no longer possible (Matzk, 1991). Therefore auxin application before pollination is disadvantageous (Matzk and Mahn, 1994).

Environmental factors such as temperature, light, and humidity have been found to be important for the rate of success in the wheat x maize systems (Matzk and Mahn, 1994). It is possible to maximize the efficiency by more suitable environmental growth conditions (22-25 °C and no moisture stress).

At an early development stage of wheat seed after pollination with the maize pollen the endosperm ceases to develop and then degenerate to form a residue. The immature embryos continue to develop although the size and shape are widely varied depending on the genotypes used (Inagaki, 1990). Later seeds begin to shrivel so that an artificial rescue of immature embryos is necessary for further development.

Embryos are excised in a sterile cabinet and transferred onto culture medium without hormones. The developmental stage of the immature embryos at the time of excision is

critical with respect to plant regeneration (Inagaki, 1985a). Matzk and Mahn (1994) suggest that the embryos should be rescued 15-18 days after pollination. Immature embryos are successfully regenerated to haploid plants at high frequency, by the excision at the suitable developmental stage of embryos whilst scutellar overgrowth seems to inhibit embryos from germinating, resulting in low plant regeneration rate (Inagaki ,1990). Most pollinated florets produce plump seeds but the seeds do not always contain embryos. Inagaki (1996) stated that 2 to 4 embryos per wheat spike on average can be obtained.

Seed set in wheat cross with pollen from the maize is somewhat smaller than in selfed seed, and seeds are filled with an aqueous solution instead of solid endosperm as found in selfed seed (Inagaki, 1996; 1990; Matzk and Mahn, 1994). Factors like fitness of the donor plant, quality of the maize pollen or damage of the stigma caused by emasculation will probably have a greater effect on success rate in haploid production method (Bitch *et al.*, 1998).

2.2.3 Production of doubled haploids (DH)

The progress in wheat haploid production through the wheat x maize technique can be obtained by improving crossing procedure for higher frequencies of seed setting and embryo culture technique for more efficient regeneration of haploid plants (Inagaki, 1996). Most of the plants regenerated directly from immature embryos are wheat euploid with 21 chromosomes. The spikes which emerge are completely sterile and will not set seeds unless treated with colchicine (Inagaki, 1990) to double the chromosome number to 42.

For artificially induced chromosome doubling, colchicines+DMSO solution can either be incorporated into culture medium, or the haploid seedlings can be submerged in the solution (Liang *et al.*, 1990). Plantlets are carefully taken out of the soil at the tillering stage, their

roots cut off to 5 cm and immersed in a solution of 0.2% colchicine + 1.5 % DMSO (Dimethy sulfoxide) for 5 hours at room temperature (Liang *et al.*, 1990). The colchicine treatment usually kills the first tiller in all genotypes and chances of recovery from secondary tillers vary with genotypes (O'Donoughue and Bennet, 1994)

Noticeable differences were shown to exist by Lefebure and Devaux (1996) in the proportion of diploid nuclei on tillers that develop after colchicine treatment. A flow cytometry analysis shows that the relative percentage of diploid over total nuclei of colchicine treated haploid plants varies revealing differences in chromosome doubling efficiency.

2.3 Performance of doubled haploids (DH)

To evaluate the performance of doubled haploids (DH) the following questions need to be answered:

(i) How much time does doubled haploid save in the plant breeding process

(ii) How homozygous and homogeneous are doubled haploids

(iii) How representative is the gametic array from doubled haploid and is gametoclonal variation present, and

(v) How do doubled haploid perform in the field

2.3.1 Time saved by using DH technique

A minimum of 2 years could be saved using doubled haploid breeding technique instead of F_5 derived lines (Bitch, 1998). The time saved will increase if the conventional breeding method

derives its lines from later generations,(F_3)` or later or decreased if the conventional breeding method derives its lines in earlier generations, F_3-F_4 (Baenziger *et al.*, 1984; Baenziger, 1996). The time saving will also decrease if the doubled haploids are derived from F_2 or later generation plants rather than F_1 plants (Baenziger, 1996). In Kenya where varieties are only released as pure lines at F_7 and F_8 a saving of over five years will be realised by use of the DH technique.

One of the frustrations of plant breeding is the wait from the time of identification of an elite line as a potential parent to the evaluation of its progeny from crosses. The speed of doubled haploid breeding allows a breeding program to respond rapidly to new parents and conditions that affect the crop (Baenziger, 1996).

2.3.2 Homozygosity and homogeneity of DHs

Studies done by Bentolila *et al* (1992) and Murignaux *et al* (1993) on homogeneity within doubled haploids in maize indicated very little variation. The genetic marker studies indicated that doubled haploids lines are extremely uniform (Beanziger 1996). In practice even in studies specifically designed to study variation that may be induced by the method used to create doubled haploid lines the resultant lines have been exceptionally uniform in the field (Snape *et al* , 1988; Baenziger et al, 1989; Baenziger, 1996; Wernsman, 1992).

There may be variation among doubled haploid lines but there is very little variation within individual haploid lines (Baenziger, 1996). The within lines variations that is found can be attributed to processes that occur after the doubled haploid lines were developed (Baenziger, 1996; Mungneux *et al.*, 1993).

2.3.3 Representativeness of DHs

In doubled haploid derived by wide hybridization distorted segregation seem to be less common (Baenziger, 1996). Results from cytological analysis in Triticale show that, at least for the chromosome constitution, the gametic variability is well represented in the plants regenerated after anther culture (Charmet *et al.*, 1986; Miao *et al.*, 1988; Henry and de Buyser 1990).

Research done on barley DH by Schon *et al* (1990), Powell *et al* (1984,1990), Boll *et al* (1989) and Kjner *et al* (1990) did not indicate any aberrant segregation ratio among the barley doubled haploid lines tested. In considering gametic arrays, it should be remembered that doubled haploids, unless they are used in a recurrent selection program should be derived from crosses where relatively few genes segregate (Jansen, 1992; Baenziger, 1996). If many genes segregate, then DH method require more DH lines than normally are available (Baenziger 1996).Doubled haploid lines can be tested using standard evaluation procedures (replicated yield trials) in multiple environment interactions of quantitative trait loci (QTL) (Baenziger, 1996).

2.3.4 Gametoclonal variation in wheat x maize wide crosses

Snape *et al* (1988) identified gametoclonal variation in wheat doubled haploids developed by either wide hybridization or anther culture but not in barley. This may be related to the polyploid nature and genetic redundancy of wheat that tolerated genetic changes more easily than barley, a diploid species (Baenziger 1996). Suenaga and Nakajima (1993) also found gametoclonal variation in wheat doubled haploid developed by wide hybridization. The variation was believed to be caused by the colchicine doubling step and not hormonal

treatment applied to florets to delay embryo abortion or the embryo rescue step (Baenziger 1996).

The amount of gametoclonal variation in a population is often biased by a few very poor lines which greatly lower the doubled haploid populations mean (Baenziger *et al* 1989c; Laurie and Snape, 1990). These lines would be easily discarded in a breeding program hence would not greatly affect breeding progress (Baenziger 1996). Doubled haploid lines are as environmentally stable as commercially licensed cultivars (Rein bergs *et al.*, 1978). The stability of doubled haploids is due to the fact that most conventionally derived cultivars have within cultivar heterogeneity because it is virtually impossible to obtain homozygous and homogeneous lines by selfing if the parents differed for many genes, (Baenziger, 1996).

Even with aberrant genetic ratio and altered gametic arrays doubled haploid have been used for efficient molecular mapping and genetic studies and should be useful in plant breeding (Baenziger 1996). In general doubled haploids developed by wide hybridization (chromosome elimination) have less gametoclonal variation for agronomic performance than those developed by anther culture.

Comparison between doubled haploids (DH) produced from same F_1 plants as lines by the pedigree, bulk and single seed descent (SSD) breeding methods were made by Choo *et al* (1984). In studying the gametic array researchers have found non-parental genes or phenotypes. These results are often explained as gametoclonal variations. Evidently the chromosome changes are small or infrequent. Gametoclonal variation can be used when it meets the breeders' objectives. For example, gametoclonal variants are believed to have increased disease resistance in tobacco (Whitespoon *et al.*, 1991) and occasionally in wheat

(Pamsi and Picard, 1986). It should be noted that gametoclonal variation is dependent upon the method of producing the doubled haploid and that every report on field results is confounded with the method of creating the doubled haploid (Baenziger *et al.*, 1989a; Baenziger, 1996).

2.3.5 Performance of doubled haploid in the field

Researchers who have worked with haploid breeding programs in barley have generally concluded that 100 DH lines from a cross are usually sufficient to obtain a superior line relative to yield (Wenzel, 1995). However, this may depend on how many traits or genes one is trying to combine in selection (Kasha, 1995). Most studies have shown that one can be quite successful in selecting recombinants from DH lines produced from F_1 (Wenzel, 1995). Where one knows that there is tight linkage of genes, one could go to F_2 to obtain an additional generation of recombinants (Kasha, 1995); however going beyond F_2 to obtain recombinants is usually not warranted (Snape, 1981).

Importance of the method of producing doubled haploid can be seen in many early studies of doubled haploid field performance. Wernsman *et al* (1989) found anther culture derived doubled haploid more generally inferior to maternally derived doubled haploid

Snape *et al* (1990) compared doubled haploid using two different wide hybridization methods, wheat crossed by *bulbosum* and wheat x maize and found the two methods to be equivalent in terms of field performance.

When compared to the traditional breeding techniques the DH method has been found to be superior.The lines derived by the bulk and pedigree methods were better populations of lines as a result of selection while SSD derived lines should be a random selection of the population derived from the cross. The DH lines should also be a random selection of gametes but require fewer lines to find the superior gametes than using SSD (two gametes per line).

The results indicated that the best DH lines from a cross were equivalent to or better than to the best lines produced by other breeding methods, the DH lines were also as stable over environment as the inbred lines produced by other breeding methods (Reinbergs *et al.*, 1978).

Kasha *et al* (1977) concluded that additive gene action was predominant for yield in some crosses so that heterotic effect could be fixed in pure lines such as DHs . Kasha (1995) agrees with Ba- Bong and Swaminathan (1995) that for traits expressing strong additive genetic effects the derived DH lines could reach the heterosis level of the F_1 hybrids. Bjornstad *et al* (1993) used means and genetic variances to compare the doubled haploid population and single seed descent population. By coupling rapid lines development with homogeneity doubled haploid breeding is ideal for countries which require high level of cultivar purity. It is also ideal for rapidly responding to changing conditions such as where new pest biotype occurs or is introduced or when cultivars are to be developed for non-traditional wheat areas (Baenziger, 1996).

The major remaining limitation to the DH technique appears to be the cost of producing doubled haploid lines in sufficient quantities for a breeding programme the same methods limit the germplasm that is available for plant breeding.

Sunint *et al.*, (1993) basing his study on rice cultivar in Japonica, Indica and Savannah types using the haploid versus the pedigree method, showed a substantial cost saving of 14-26% when using the DH procedures. Most of the saving resulted from the three fewer generations of inbreeding when using haploids. But Kasha *et al* (1995) points at that many factors have to be considered e.g. equipment depreciation, salaries and time, cost of supplies, and saving because of the fewer generations required to reach homozygosity with haploids, hence less cost.

CHAPTER 3

3.0MATERIALS AND METHODS

3.1 Development of F_1s in wheat crosses

Crossing three drought tolerant lines namely; Duma, Kenya Mbweha and Ngamia as female with two high yielding commercial varieties, namely Kwale and Kenya Chiriku as males, produced six F1 hybrids. A brief description of parents involved in the crosses is given below (KARI, 1997):

Duma – is an early maturing, low yielding and drought tolerant variety. It is susceptible to yellow rust. Under proper management it can yield over 1.9 tons per hectare in dry areas. It is a Semi-hard grained wheat variety with white kernels. Its ears are tapered, awned and have cream chaff.

Ngamia – is an early maturing variety, which is drought tolerant but low yielding. Under proper management it can yield as high as 2.0 tons/ha. It is susceptible to yellow rust, does well at altitudes below 1800 metres above sea level in dry areas. It has reddish brown chaff, red kernels with glaucous neck.

Kenya Mbweha -is a drought tolerant variety late maturing with average yield of 2.5 tons/ha under fairly dry conditions. It is resistant to yellow rust. It has creamish chaff, awned and tapered ears. The kernels are white.

Kenya Chiriku- is an early maturing commercial variety for conventional wheat areas. It is high yielding ranging between 3.2 to 7.0 tons/ha under good environment and

management. It is resistant to yellow rust but susceptible to drought. It is a short wheat with awned spikes which are tapered. It has cream chaff and the kernels are red.

Kwale- is a late maturing high yielding commercial variety. Yield ranges between 2.9 and 7.0 tons/ha under good environment. It is susceptible to drought and yellow rust. It is high quality wheat for baking. The grains are hard and red. It is a wide adapted variety with reddish brown chaff. The heads are awned and tapered.

All the crosses were made in the cage at the National Plant Breeding Research Center, Njoro during the months of May to September 1998. The crosses were made as shown below.

Male Female	Kwale	Kenya Chiriku
Duma	X	X
Ngamia	X	X
Kenya Mbweha	X	X

X represent crosses.

Crosses were made following the go-go method outlined by Kinyua (1997). The emasculation was done on the female plants without injuring the stigma before the anthers matured, while they were still green and in the florets. The heads emasculated were covered with glycine bags left for two to three days depending on the weather conditions; during cold conditions the time was extended to four days. After the stigma matured, heads were selected from respective males to be crossed with each female. Pollination was performed by trimming the glycine bag at the top and the male head shaken back and forth to shake off the

pollen grains which then pollinated the emasculated female heads. The F_1 seeds were harvested at maturity separately.

3.1 Production of haploid plants by crossing F_1s with maize

The F_1 seeds were planted in pots and provided with light for 16 hours a day and temperature maintained at 21-25 ° C. The soil mixture was peat soil mixed with sand and Diamonium Phosphate (DAP) fertilizer (2 g/2kg pot) Some gravel's were placed at the bottom of the pot to facilitate drainage. The Hybrid 512 maize seeds had earlier been planted so as to synchronize flowering with that of wheat F_1s.

At ear emergence, wheat spikes were emasculated following the removal of the apical and basal spikelets followed by theremoval of central florets of each spikelet. The glumes were not clipped with the awns as is the case with normal crossing emasculation process but the awns were trimmed to the level of the glume. The emasculated spikes were then enclosed with polyethylene bags to maintain high humidity after emasculation. One day before predicted anthesis, emasculated spikes were then pollinated with freshly collected maize pollen. The pollen was collected by picking mature anthers on petri dishes, and once the anther burst to release the pollen a soft brush was used to brush the wheat spikelets with the pollen. Care was taken not to damage the stigma. On two consecutive days after pollination, the uppermost internodes of wheat culms with pollinated spikes were needle –injected with a 100mg/l 2, 4-D solution daily.

3.3 Haploid embryo culture.

After about 14 days of spike growth on wheat plants, wheat caryopsis were removed from spikes and sterilized in a solution of 2% sodium hypochlorate and few drops oftween 20 for

15 minutes. After rinsing twice with sterile distilled water, embryos were aseptically excised and transferred onto half strength Murashige and Skoog medium supplemented with 20g/l sucrose and 8g/l agarose (Inagaki 1996) in petri dishes. These were then placed in the fridge at 4° C for three days after which they were incubated at 25°C in the dark for five days and then in the light using 16 hr day length and Ca. 5000 Lux light intensity. The composition of the media is shown in appendix 1.

3.3.1 *Ex-vitro* growth of haploid plantlets

Cultured plants with fully developed roots and leaves were transplanted to pots and grown further. This was done in the green house where all the conditions were set for fast growth and tillering. The temperature was maintained at 21 ° C -25 ° C and day length at 24 hours. The chromosome number was examined in squashed preparation of root tips stained with accetocarmine. The size of the stomata was used to determine the ploidy level of the plants.

3.4 Chromosome doubling of haploids

At tillering the plants in the pots were watered before they were removed and washed. The roots were cut to about 2 cm below the crown and the leaves were reduced. Plants were then immersed in colchicine solution (0.2% colchicine, 2% dimethyl sulfoxide (DMSO) and 15 drops tween-20) in a flask for 3 hours at room temperature. The solution was occasionally stirred to increase absorption by the plants. The plants were then washed thoroughly with running tap water for 5 hours. These were then repotted using forest soil and grown under favorable, tillering enhancing conditions. The Temperature was maintained at 20-25 ° C, humididy was saturated and with 16 hours day length.

3.5 Screening for drought tolerance

Twenty DH lines and their five parents were screened at the rain-out shelter at National Plant Breeding Research Center (NPBRC), Njoro. They were planted in a randomized complete block design (RCBD) with three replications. Each plot was 1 metre long with two rows 20 cm apart. A 50 cm-path separated the blocks while interplot space was 30 cm. Five seeds were planted per row due to the few number of seeds available DAP fertilizer was applied at planting at the rate of 150 kg/ha. The drip irrigation was done to simulate 200 mm of rainfall during growth period in dryland. The watering was distributed such that 50 mm were applied at seedling stage, 50 mm after tillering, 50 mm after booting and 50 mm after anthesis or in three weeks intervals. The pedigree of the DH's is shown in Table 4.

3.5.1 The Site

Njoro is situated 20 km southwest of Nakuru town at 2,160 metres above sea level longitude 45^0 East. It is in the Agro-ecozon 3. The soils in Njoro are deep well drained, dark brown, andosols with high fertility. It is classified as wheat/maize and barley zone. The average annual rainfall is 930 mm. Yearly average temperature is about 17-22° C with mean maximum temperature ranging from 25° C to 30° C and mean minimum of 7°C to 10°C. The cropping period for Njoro is March to August and November to February.

3.5.2 The Rain-Out Shelter

The rain shelter is a metal frame erected in rails that are resting on quarry stones held together by concrete. The roof is covered with translucent sheets and foldable polyethylene sheet on the sides. Drip irrigation sets run along the rain-out shelter area 40cm apart with the delivery point spaced at 30 cm. The pressure of the water throughout the system is automatically

graduated to deliver equal amount of water. The length of the rail is 30 m while the shelter is 15 metres long. The shelter therefore rests on half of the runway. The width of the shelter is 7 metres. 3.5.3 Data collection

Data on percent germination, number of tillers at anthesis, plant height at maturity, number of effective tillers, length of the spike , number of kernels per spike, weight of 10 seeds (g) were collected and analyzed biometrically. The best lines were recommended for evaluation for quality characteristics such as kernel colour, texture and protein content. A detailed procedure of parameter measurement is given below.

3.5.3.1 Percent emergence

The emergence was recorded one week after planting. Total number of seedlings that emerged was compared to the number of seeds planted per row as a percentage.

3.5.3.2 Number of tiller at booting

The number of tillers per plant was counted for all the plants at 50% booting and then the average noted.

3.5.3.3 Days to 50% anthesis and physical maturity

This was taken as the number of days that plants took to reach anthesis and physical maturity. The anthesis time was taken when 50% of the plants had flowered while physical maturity was when all the plants had turned golden yellow and the grain s hard and solid

3.5.3.4 Number of effective tillers

This was the number of tillers which had seed bearing heads. The number of tillers with heads were counted and their average taken.

3.5.3.5 Ear length

The ear length on all the primary tillers were taken in each plot at maturity and averaged. The measurement was taken from the neck to the tip of the last floret.

3.5.3.7 Number of spikelets per head

The number of spikelets per head was taken for all the primary spikes at maturity and then averaged.

3.5.3.6 Number of sterile spikelets per head

This was the average sterile spikelet per head. All the empty spikelets on the primary tillers were counted and then averaged

3.5.3.8 Number of grains per spike

The number of grains per head was the average number of grains of all the primary tillers in each plot at physiological maturity and averaged over the spikes.

3.5.3.9 10- kernel weight (g)

This was obtained by counting ten grains from each plot and their weight taken in grams.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Doubled Haploids Production

4.1.1 F_1 wheat crosses

Between 200 and 400 florets were hand emasculated and pollinated in each of the six different crosses made (Table 1). In case of crosses Duma x Kwale and Duma x Kenya Chiriku 200 florets were emasculated and pollinated. About 300 florets were emasculated and pollinated in the cross Ngamia x Kenya Chiriku.

The success of crosses in the three drought tolerant varieties and the two high yielding varieties was similar. The seed set varied between 40% and 55% with a mean number of seeds harvested per cross of 49.44%. The highest success was obtained in crosses between Kenya Mbweha and Kenya Chiriku in which 55 % of F_1 seeds were produced. Ngamia x Kwale had the lowest seed set (40 %). These compares well with work of Kinyua (1991).

A total of 292 seeds were planted into potted soil and heads from these were pollinated with the maize pollen. In general the embryo rescue of F_1 was lower than In vivo process where the seeds are left to develop to maturity when still on the mother plant. Having an average of 44.9 % plantlet transplanted in this study was lower than the average germination percentage (70-95 %) (Acland1980). This difference was expected given the *in vitro* process and the number of shocks the embryo underwent from harvesting to rescuing.

Table 1. Number of F_1 seeds obtained in six different wheat crosses.

Cross (F_1)	No. of florets pollinated	F_1 seeds harvested	
		No.	(%)
Duma x Kwale	197	97	49
Duma x Chiriku	198	99	50
K.Mbweha x Kwale	402	201	50
K.Mbweha x K.Chiriku	399	219	55
K. Ngamia x Kwale	301	121	40
K.Ngamia x K. Chiriku	303	149	50
Total	1800	886	49

4.1.2 Haploid production and plantlets regeneration

A total of 145 F_1 spikes were pollinated with the maize pollen as shown in Table 2. Of the 2,880 florets pollinated with the maize pollen 413 swollen caryopsis were harvested. This was 14.34 % success. On average 3.014 caryopsis per head were harvested. This rate of success was comparable to those reported by Bitch et al (1998) who found the mean number of embryos between 21.5 % and 16.5 %. Inagaki et al (1998) working with Durum wheat had a success rate ranging between 0.0 % and 15.8 % in embryo formation and 2.6 % and 84.8 % in caryopsis formation.

The caryopsis formation ranged between 10.0 % and 25 % in this study. The cross Ngamia x Kwale had the highest success of 25 % caryopsis formation while Kenya Mbweha x Kenya Chiriku had a lower rate of 10.0 %. This range agrees with those of Matzk and Mahn (1994) who had mean caryopsis formation between 23 % and 38 %. Of the 413 caryopsis 57 embryos were excised making 13.9 % and 1.979 % of the total number of florets pollinated. This was an average of 0.4 embryo per head pollinated. This was lower than what has been reported by Inagaki (1996), Kinyua (1999), Matzk and Mahn (1994) and Bitch (1998) but was similar to the work of Inagaki (1998) whose most of the varieties had a mean of 2 % embryo formation. The average number of embryo production was 2 per spike although this was different between wheat genotypes. This had also been reported by Inagaki (1996; 1998).

Most of the insolated embryos grew and survived the tissue culture up to transplanting. The average success was 86 %. The crosses involving Duma had 100 % success while the crosses Kenya Mbweha x Kwale had 70 % plantlets formation. A total of 49 plantlets were developed from the 57 embryos isolated. This makes 11.86 % of the total number of caryopsis excised. This compares well with results reported by Bitch *et al* (1998) who had 10.1 % of

the florets pollinated. The low number of embryo isolated can be attributed mainly to temperature fluctuations (Inagaki, 1996). The embryos can be lost at tissue culturing point if the conditions are not right.

In this study the genotypic influence of the wheat parent and heterozygosity of the wheat in haploid production was not very distinct (table 2). Quite a number of factors can influence the embryo formation in addition to the wheat genotype. These include the maize genotype (Bitch *et al* 1998), method of emasculation (Kinyua 1999), Time of pollination (Inagaki 1996) time of 2,4-D application (Wedzony and Van Lammeren, 1996), time of embryo rescue (Inagaki, 1996 Laurie and Bennett 1988). Other environmental factors such as the humidity and temperature during the growth and flowering of the mother plant are important (Inagaki 1996). The polyethylene bag was used to maintain high humidity around the head at pollination. The temperature was maintained at an average of 22° C during the day and 17° C at night. This was the optimum temperature as suggested by Inagaki (1996). All the plantlets transplanted survived to tillering stage and were examined for ploidy level before colchicine treatment was done.

Table 2. Number of haploid embryos and plantlets produced in Cross F_1 wheat x maize crosses.

No. of Cross (F_1)	No of florets pollinated with maize	Caryopsis harvested		Embryos isolated	(% of caryopsis)	Plantlets transplanted	
		No.	(% of florets)	No.		No.	(% of embryos)
Duma x Kwale	301	58	19.3	16	27.6	16	100
Duma x Chiriku	299	61	20.0	6	10	6	100
K. Mbweha x Kwale	802	102	12.5	10	10	7	70
K. Mbweha x K. Chiriku	798	79	10	5	6.5	4	80
K. Ngamia x Kwale	282	71	25	10	14.3	8	80
K. Ngamia x K. Chiriku	398	46	11.3	10	22.2	8	80
Total	2880	417	14.4	57	13.9	49	86

4.1.3 The ploidy level and response to colchicine treatment of the haploids

Table 3 shows the ploidy level of the plantlets produced from the crosses made with maize. Of the 16 Duma x Kwale derived haploids 11 were haploids while 5 were diploids. A total number of 40 out of the 46 plants were haploids, all the other crosses apart from Duma x Kwale and Ngamia x Chiriku where we had 5 and 1 diploids respectively; were haploids with 21 chromosomes. The size of the stomata was used to determine the ploidy level of the plantlets. Haploid plants had stomata that were half the size of the diploid plants.

The response to colchicine treatment is also shown in Table 3. Of the 40 plants treated with colchicine 27 survived the treatments. This was 56 % success. Similar results were reported by Lefebure and Devaux (1996) who found an average response to colchicine treatment of between 36 and 68 %. The success of doubling the chromosome number of haploids by colchicine treatment can only be verified if the plants are able to produce viable seeds. Of the 27 plants which survived colchicine treatment 24 or 89 % were able to produce fertile seeds. The response to colchicine treatment in crosses Ngamia x Kwale and Duma x Kenya Chiriku were the best with all the haploids that survived the colchicine treatment produced viable seeds. Duma x Kwale haploid were the poorest in surviving the colchicine treatment. This could be attributed to a number of factors. The plants could have escaped the ploidy level test where most diploids were eliminated and the plants which never formed seeds might have had abnormal polyploidy.

The effect of the genotype on the number of plants that produced viable seed was highly significant, with the cross Ngamia x Kenya Chiriku having 2 out of 6 surviving plantlets while the cross Ngamia x Kwale had 6 out of 6 plantlets surviving all the way to seed productions (Table 4). The high number of seeds developed from the doubled haploids could

be attributed to the time of colchicine treatment. Unlike the case of Inagaki (1996) where plantlets were treated at the third tillering stage, the plantlets in this case were treated at an earlier stage before tillering. This meant that all the tillers that formed were diploid. The wheat genotype also had an influence on seed set of the doubled haploids (Table 4). All the Kenya Chiriku derived DHs had a higher number of seeds harvested while the Kwale derived DHs had the lowest seed set.

Studies have been aimed at enhancing the embryo formation frequency by choice of proper crossing procedures and improvement of pollination in embryo development has been sought by use of post pollination hormonal treatment (Bains et al 1997). Culture media manipulations have been attempted for increasing the regeneration frequency of embryos. Such considerations had high influence on the high survival rate of haploids. Selfed seeds' embryos were avoided at the excising stage since they are easily identified by the presence of endosperm as reported by Suenagy (1997) and the probability of contamination by the embryos originating from selfing among the presumed haploid embryos in the filled caryopsis were very low.

Table 3. Ploidy level of wheat plantlets and effect of colchicine on haploid plantlets of different wheat crosses.

F_1 hybrid	Number of plants	No of chromosome 21	42	Plants treated with Colchicine	Colchicine survival %	Fertile heads %
Duma x Kwale	16	11	5	11	37.5	83.3
Duma x K. Chiriku	6	6	0	6	50	100
K. Mbweha x Kwale	7	7	0	7	100	85.7
K. Mbweha x K. Chiriku	4	4	0	4	75	66.7
K. Ngamia x Kwale	6	6	0	6	100	100
K. Ngamia x K. Chiriku	7	6	1	6	57	50
Total	46	40	6	40	59	89

4.1.4 Doubled haploid (DH_1) production

Table 4 shows the various DHs produced, their pedigree and the number of seeds produced per DH line. Some DHs that had less than 20 seeds were discarded at this level. The mean number of seeds per DH was 66.45. This was double the number of seeds per DH what was observed by Inagaki (1996).

The best DH in seed production was $DH_1$12 with 142 seeds followed by $DH_1$1 with 130 seeds. The DH ($DH_1$13) had the lowest number of (31) seeds. In general seeds were much smaller than either the parents or the F_1 seeds. The total number of seeds was affected by the number of effective tillers. The number of tillers was determined by the stage at which colchicine treatment was done and genotypic ability of the plants to tillering.

Table 4. The parentage of the 20 DH_1 lines and the number of seeds produced per line.

DH_1 line	Parentage	Number of seeds
$DH_1$1	Kenya Mbweha x Kwale	130
$DH_1$2	Ngamia x Kwale	35
$DH_1$3	Ngamia x Kwale	35
$DH_1$4	Duma x Kwale	45
$DH_1$5	Duma x Kwale	62
$DH_1$6	Duma x Kwale	39
$DH_1$7	Kenya Mbweha x Kenya Chiriku	103
$DH_1$8	Kenya Mbweha x Kwale	64
$DH_1$9	Duma x Kwale	84
$DH_1$10	Ngamia x Kwale	70
$DH_1$11	Duma x Kenya Chiriku	72
$DH_1$12	Duma x Kenya Chiriku	142
$DH_1$13	Kenya Mbweha x Kwale	31
$DH_1$14	Kenya Mbweha x Kenya Chiriku	54
$DH_1$15	Ngamia x Kwale	48
$DH_1$16	Ngamia x Kwale	62
$DH_1$17	Ngamia x Kenya Chiriku	70
$DH_1$18	Duma x Kwale	53
$DH_1$19	Kenya Mbweha x Kwale	64
$DH_1$20	Ngamia x Kenya Chiriku	66

4.2 General Performance of The DHs and Their Parents on drought

4.2.1 Seedling Emergence percentage

The percent emergence of the 20 DHs and their parents was significantly different at 5 % level (Table 5). Kenya Chiriku was the best in emergence with a mean of 86.67 % emergence (Table 6). The poorest parent was Kwale with a mean emergence of 36.6 %. The remaining parents had a mean emergence above the overall mean of 43.07 %. This was a wide range of variation ranging between 13.33 for $DH_1$5 to 70.0 % for $DH_1$15. Four DHs namely $DH_1$15, $DH_1$1 $DH_1$12 and $DH_1$14 were not significantly different from Kenya Chiriku in relation to seed emergence percentage.

The LSD means separation categorized the treatments into 9 groups. The poorest group included Kwale, $DH_1$8, $DH_1$19, $DH_1$10, $DH_1$13, $DH_1$16, $DH_1$5 $DH_1$18 and $DH_1$11 and all had a mean emergence percentage below the overall mean. The best DH ($DH_1$15) had a mean of 70 % emergence while the poorest ($DH_1$5) had a mean emergence percentage of 13.33 % (Table 6). The Kenya Mbweha x Kwale cross had three DHs derived from it. Two of the DHs namely $DH_1$8 and $DH_1$19 had a mean seedling emergence below that of both parents at 26.67 % and 23.33 %, respectively. This was significantly lower than Kenya Mbweha which had a mean seed emergence of 63.33 % but not significantly different from Kwale which had a mean of 36.67 %. $DH_1$1 with a mean emergence of 66.67 % was higher than the mean of the two parents but only significantly so with Kwale.

Table 5. The mean sum of squares and overall means for yield components for twenty DHs and their parents measured during the screening for drought tolerance at Njoro under the rain-out shelter.

Source	Emergence (%)	Tillers at booting	Days to50% anthesis	Effective tillers	Spikelets per head	Sterile pikelets per head	Length of head	Days to maturity	Grains per head	10 Kernel weight
Replicate	265.33	2.326	9.941	1.233	4.724**	0.0049	3.04	7.94	76.47	0.001
Treatments	1056.1**	4.814	168.77**	4.36 **	10.67**	1.66**	2.08	675.08**	121.67**	0.009**
Mean	43.07	4.49	10.59	3.474	14.57	1.93	7.41	142.5	31.95	0.32

** - Significantly different at the $P \leq 0.05$ level.

Of the two DHs produced from the Kenya Mbweha x Kenya Chiriku cross, $DH_1$7 with a mean emergence percentage of 36.67 % was significantly lower than the Kenya Chiriku (86.67 %) parent at 36.67 % but not with the Kenya Mbweha parent (63.33 %). The other DH ($DH_1$14) was also lower than both parents but not significantly (Table 6).

The 6 DHs developed from the cross Ngamia x Kwale showed a range of variability as compared to their parents. The best had emergence percentage of 70 %, which was significantly higher than the Kwale parent for the Ngamia parent (50 %) (Table 6). $DH_1$10 had the lowest emergence percentage at 20.0 %. The other DHs had their means falling between the means of the two parents.

The two DHs developed from the Ngamia x Kenya Chiriku had their mean emergence between the means of the two parents (Table 6). There was no significant difference between the DHs and their parents. Five DHs developed from the Duma x Kwale cross namely $DH_1$4, $DH_1$5, $DH_1$6, $DH_1$9 and $DH_1$18. As shown in Tables 6, $DH_1$5 and $DH_1$18 were the lowest with in their emergence percentage and significantly lower than the parental means (Table 6). The other three DHs are not different from their parents' means. The overall mean of the DHs was significantly lower than that of the parents. Of the two DHs developed from Duma x Kenya Chiriku cross, $DH_1$1 was significantly lower than the parent but $DH_1$12 was similar to the parents.

These results on emergence indicate that most DHs were lower in emergence than their parents. This can be explained by the fact that the seeds harvested from the doubled haploid plants were weaker due to the number of stresses (haploid production and colchicine treatment) the plants underwent during the development process of the doubled haploid.

Table 6. Mean performance for agronomic parameters of 20 DHs as compared to their parents tested for drought tolerance under the rain-out shelter at Njoro.

Parents	DH lines	% emergence	No. of tillers at booting	Days to 50 % Anthesis	Effective Heads	Spikelets/ Head	Length of head	Sterile Spikelets	Grains/ head	weight of 10 grains
K. Mbweha		63.33	5.00	69.67	5.0	15.333	7.500	2.000	24.333	0.367
Kwale		36.66	4.21	79.87	2.16	14.533	7.593	3.994	27.936	0.306
K.Mbwehe	$DH_1$1	66.67**	5.33	84.67♦	4.00♦	16.000**	8.500	2.00*	34.00	0.301
X	$DH_1$8	26.67♦	5.71	84.87♦	3.67♦	17.533**	7.593	2.494*	35.936	0.317**
Kwale	$DH_1$19	23.33♦	3.71♦♦	79.867	3.66	15.533	7.593	1.494*	32.936	0.27♦
K. Chiriku		86.67	4.33	64.33	2.00	15.333	7.000	3.000	30.667	0.312
K. Mbwehe	$DH_1$7	36.67♦	7.39**	64.11	6.11**	19.301	5.704	2.009	43.489***	0.282
XK. Chiriku	$DH_1$14	56.67	2.67♦♦	70.0	2.33	17.333	7.167	1.333*	36.667**	0.343
Ngamia		50.0	3.67	68.667	3.00	10.00	6.667	1.667*	41.667	0.349
	$DH_1$2	40.0	4.39	89.11♦	4.11	19.301**	6.204	1.009*	41.489**	0.336
	$DH_1$3	40.0	5.21	74.864	3.66	16.033**	7.843	1.995*	24.94♦	0.21♦
Ngamia	$DH_1$10	20.0♦	4.33	75.33	2.00♦	13.333	6.167	1.006*	27.67♦	0.346
X	$DH_1$13	33.3	4.30	76.94	3.95	15.349**	8.147	1.996*	41.755**	0.304
Kwale	$DH_1$15	70.0*	3.33♦	64.00*	3.33	15.333**	6.833	0.67***	30.00*	0.367
	$DH_1$16	30.3	8.39**	79.119	6.1***	19.301**	8.704	0.09***	28.489	0.304
Ngamia X	$DH_1$17	46.67	0.803♦♦	80.61♦	0.45♦♦	13.765	10.482	4.980♦♦	4.33♦♦	0.12♦♦
K. Chiriku	$DH_1$20	40.0	5.00	67.33	4.33	14.000**	6.667	1.667**	28.67♦	0.19♦♦
Duma		66.67	6.33	58.33	3.00	15.000	8.333	2.000	28.333	0.467
	$DH_1$4	26.67	2.80♦♦	69.44	2.45	13.349	6.148	2.996	19.255	0.305♦
Duma	$DH_1$5	13.33♦	3.39	82.12♦	3.11	19.301	9.704	2.009**	35.489	0.310♦
X	$DH_1$6	50.0	7.39	58.19*	2.89	14.617	6.759	1.510**	33.308	0.367♦
Kwale	$DH_1$9	50.0	7.00	64.00*	4.00	11.333	8.333	2.333**	29.333	0.266♦
	$DH_1$18	23.3♦	4.30	60.44*	2.45	12.349	6.147	1.996**	32.255	0.326♦
Duma	$DH_1$11	16.67♦♦	3.71♦	66.366	2.16	14.533	7.593*	0.995	40.936*	0.307*
X	$DH_1$12	40.0	5.67	84.667♦♦	3.67	17.333	8.33***	2.000	44.00**	0.421**
K. Chiriku										

* Significantly higher at 5 % level than the lower parent

** Significantly higher than the mean of the parent at P≤0.5

♦ Significantly lower than the higher parent at 5 % level.

♦♦ Significantly lower than the lower parents at 5% level

4.2.2 Tillers at booting

There was significance difference between the DHs for the number of tillers at booting. Except $DH_1$17, which had, significantly low number of tillers at booting and $DH_1$7 and $DH_1$16 which had a higher number of tillers the other DHs were not significantly different between themselves and within crosses.

Most of the DHs had similar number of tillers at booting as their parents. The overall mean number of tillers at booting was 4.49 with most of the DHs means being distributed around it. Of the three DHs developed from Kenya Mbweha x Kwale one had a mean number of tiller of 3.71 (Table 6), which was significantly, lower than the parent Kenya Mbweha at (5.00). The other 2 DHs had higher means than the average of the parents. The DH_7 had a significantly higher number of tillers than both the parents at 7.39%, while DH114 developed from the same cross had a significantly lower number of tillers (Table 5 and Table 6).

For the 6 DHs developed from Ngamia x Kwale, $DH_1$16 had a mean of 8.39 which was significantly higher than both parents. DH115 with a mean of 3.33 was lower than Kwale. The other DHs had their means falling between the means of the two parents. This was a normal distribution around the mean number of tillers at booting of the two parents (Table 6).

For the two DHs developed from Ngamia x Kenya Chiriku cross, $DH_1$17 had the lowest mean number of tillers of 0.803 while $DH_1$20 had a mean number of tillers of 5.00 which was not significantly different from that of the parents (Table 5). The results are mixed for the DHs developed from the Duma x Kwale cross. $DH_1$4 and $DH_1$5 had lower number of tillers compared to average of the two parents while $DH_1$6 and $DH_1$9 with mean 7.39 and 7.00 respectively were higher than the means of the parents (Table 6). The two DHs developed

from Duma x Kenya Chiriku were showed a wide range of difference. $DH_1$11 was significantly lower than the parents while $DH_1$12 fell between the means of the two parents (Table 6). From the two Tables (5,6), it is evident that most of the DHs had tillers closer to the mean of the two parents apart from $DH_1$17 and $DH_1$20 that were lower $DH_1$7 and $DH_1$16 that were superior.

4.2.3 Days to 50 % anthesis

The early varieties included Duma, Ngamia and Kenya Chiriku. This was in agreement with the findings by Kinyua (1997). The Doubled Haploids $DH_1$14, $DH_1$20, $DH_1$11, $DH_1$7, DH_1 9, DH_1 18 and $DH_1$6 had significantly lower number of days to anthesis as compared to their parents. $DH_1$14, $DH_1$20, $DH_1$11 and $DH_1$7 were all developed from crosses with Kenya Chiriku as one of the parents. This suggests that Kenya Chiriku contributed the genes for earliness. The DHs with Ngamia as one of the parents, apart from DH_{15} took longer to to reach anthesis. The Kenya Mbweha x Kwale derived DHs took equally long to reach anthesis as their parents. The results indicate that days to anthesis are highly heritable.

The early parents (Duma, Ngamia) in this study were also drought tolerant. Such wheats tend to head when exposed to drought (Guinta et al 1993). Ten of the DHs had their days to anthesis between the means of their two parents. This indicates that they combined some characteristics of both the early maturing varieties as well as those of the late maturing varieties.

The ANOVA (Table 6) shows that there was significant difference between the DHs and their parents in days to 50% anthesis. The DHs fell into three categories (early as the early parents, between the two parents or as late as the late parents) but very few DHs fall outside the

anthesis period of the two parents. The three DHs namely $DH_1$1, $DH_1$8 and $DH_1$19 produced from the Kenya Mbweha x Kwale F_1 were either later than the early parent or similar to the late parent. $DH_1$1 with a mean number of days to anthesis (DA) (84.667) and 167 days to physiological maturity (DPM) was significantly later than the early parent, Kenya Mbweha (69.667 DA and 138 DPM) but not significantly different to the late parent (79.867 DA and 161 DPM). $DH_1$8 was also significantly later in anthesis than Kenya Mbweha (84.867 DA and 167 DPM). $DH_1$19 took (79.867 DA and 168 DPM) to anthesis and was not significantly later than either parent although later than Kenya Mbweha.

The two DHs derived from the Kenya Mbweha x Kenya Chiriku cross were not significantly different from their parents in number of days to anthesis. $DH_1$2 derived from Ngamia x Kwale, was significantly earlier than Ngamia which is an early maturing variety while $DH_1$15 was significantly earlier than Kwale which is a late maturing variety. The other four DHs (Table 6) fell between the means of their parents and were not significantly different from either of the parents. For the two DHs developed from the Ngamia x Kenya Chiriku cross $DH_1$17 took 80.613 to anthesis and were later than both parents. Although developed from a late and an early maturing varieties the DH took more to attain maturity than the late parent. $DH_1$20 had mean days to anthesis between the two parents.

All the DHs except $DH_1$5 developed from Duma x Kwale F_1 were significantly early in reaching anthesis than the late parent. $DH_1$5 was significantly later than Duma, which is an early maturing variety. $DH_1$4 with a mean number of days to anthesis at 69.44 fell between the two parents, Duma (58.33 days) and Kwale (79.87days).

Although $DH_1$12 was devived from two early maturing varieties it showed late maturing characteristics by reaching 50% anthesis on the 81st day and maturity to 169th day. This was significantly higher than for both parents. With 66.67 days to anthesis and 133 days to

physiological maturity which is between that of the two parents $DH_1$11 falls under the category of early varieties.

These two characteristic (days to 50 % anthesis and physiological maturity) had a high variation making them important in selection for maturity period. This is also an indication of the character quantitative and being controlled by many additive genes.

4.2.4 Effective Heads

Seven of the DHs ($DH_1$14, $DH_1$8, $DH_1$19, $DH_1$4, $DH_1$6, $DH_1$18 and $DH_1$11) had their mean number of effective tillers falling between the means of the two parents. Other DHs such as $DH_1$7 and $DH_1$16 had a mean number of effective tillers higher than that of the two parents (Table 6). The other DHs had mean number of effective tillers below the mean of the two parents but equal to the mean of the lower parent.

With a mean of 3.47, most of the DHs showed higher number of effective tillers than the parents. There was a negative correlation between the number of effective tillers and tillers at booting. Those DHs which had many tillers at booting had few of their tillers being effective. Those with few tillers at booting had most of their tillers being effective. Most of the DHs which were affected by drought most at the seedling stage responded by producing a hig number of tillers when water was availed but were not able to form effective tillers with seed bearing heads. An example is $DH_1$17 which had 10 tillers at booting but only one tiller produced a head with seeds.

All the three drought tolerant varieties had a significantly high number of effective tillers compared to the high yielding drought susceptible parents. The DHs had their mean number of effective tillers falling between the means of the two parents.

In most of the DHs the number of effective tillers were higher than that of either one parent or both parents. The three DHs developed from Kenya Mbweha x Kenya Chiriku were higher than the lower parent. Of the three, only $DH_1$7 had higher number of effective tillers than both parents but only significantly so with Kenya Chiriku which had a mean of 2.00 effective tillers/plant (Table 5).

All the DHs developed from the Ngamia x Kwale were higher than the lower parent apart from $DH_1$15, the rest developed from Ngamia x Kwale. Superiority was shown by $DH_1$16 showed significantly higher number of effective tillers than both parents (Table 6). The two DHs developed from Ngamia x Kenya Chiriku differed in their formation of effective tillers. $DH_1$17 had a significantly lower number of tillers than the parents, while $DH_1$20 had a higher number of effective tillers above the mean of the two parents. (Table 6)

The number of effective tillers formed by all the DHs derived from the Duma x Kwale, were not significantly different from that of their parents. The other three DHs had their means falling between the means of the two parents. The two DHs($DH_1$11and $DH_1$12) derived from Duma x Kenya Chiriku had their mean number of effective tillers between the means of the parents.

4.2.5 Length Of Head

There were differences between individual treatments (Table6). The length of the head of most DHs was not significantly different from that of the parents. $DH_1$17 had the longest head of (10.48 cm), which was significantly longer than that of both parents significantly at 5% level. The head length determines the number of grains. The length also is determined by the ability of the plant to withstand drought at booting and heading period.

4.2.6 Spikelets per head

The overall mean spikelets per head was 14.57 (Table 6). Five DHs namely $DH_1$5, $DH_1$7, $DH_1$12, $DH_1$14 and $DH_1$16 had significantly more spikelets than the other DHs.

The number of spikelets per head can be used as basis to selection for drought tolerance. The higher the number of spikelets the more is the degree of tolerance during the booting stage. This is an indication that the entries were able to produce a long head even under stress at booting when the length of head was determined. Most of the assimilates must have been directed to the spikelets (floral parts) formation during this period. Stress imposed at seedling establishment stage and at booting reduced the number of spikelets per head. The highest parent, Kenya Chiriku had a mean number of 15.33 spikelets per head, while the heghest DHs had a mean number of 19.30 spikelets (Table 6).

4.2.7 Sterile spikelets per head

There was a wide range of variation in spikelets sterility among DH lines as indicated by the high cv. of 37.07 (Table 6). The $DH_1$16 had a minimum of sterile spikelets (0.09) while $DH_1$17 had the highest sterility of 4.98. Kwale and Kenya Chiriku had significantly higher number of sterile spikelets (3.99 and 3.00 respectively) as compared to the drought tolerant varieties. This was expected given that the two are high yielding varieties. under low moisture content the plants are unable to fill the grains.

Eight DHs showed a lower number of sterile spikelets compared to their parents. These included $DH_1$15, $DH_1$11, $DH_1$10, $DH_1$2, $DH_1$14, $DH_1$19, $DH_1$16 and $DH_1$20 which had significantly lower number of sterile spikelets per head than those of their parents.

This is an expression of hybrid vigour in the DHs lines for fertility than their parents during drought stress condition. Similar results have been obainted in other plant (Inagaki, 1996).

The other DHs were either higher than the lower parent or in a few cases lower than both the parents.

Most of the DHs had spikelets sterility within the range of the two parents. Only $DH_1$17 had more number of sterile spikelets than both of the parents (Table 6).

The DHs produced from the cross Kenya Mbweha x Kwale had their mean number of sterile spikelets between the mean of the parents. The three DHs were significantly lower than the Kwale parent, which had a mean of 3.99 sterile spikelets per head. $DH_1$8 at 2.00 spikelets per head was as good as the higher parent while $DH_1$19 at 1.49 sterile spikelets per head was lower than the two parents.

Of the two DHs developed from the Kenya Mbweha x Kenya Chiriku $DH_1$14 had a mean of 1.33 sterile spikelets per head which was significantly lower than Kenya Chiriku. $DH_1$7 was not significantly different from the two parents.

All the 6 DHs produced from the Ngamia x Kwale were significantly lower in in sterile spikelets than the Kwale parent. $DH_1$15 and $DH_1$16 were significantly higher than the two parents. $DH_1$2 and $DH_1$10 had lower number of sterile spikelets than both the parents but not significantly different from Ngamia. $DH_1$3 and $DH_1$13 had their mean sterility between the two parent.

There was a wide range of difference for the two DHs produced from the Ngamia x Kenya Chiriku F_1. $DH_1$17 had a mean sterility higher than both the parents while $DH_1$20 had its mean significantly lower than Kenya Chiriku but similar to Ngamia.

Of the five DHs development from Duma x Kwale F_1 only one, ($DH_1$4) was similar to the two parents. $DH_1$6 and $DH_1$9 had their means between the two parents but significantly lower than Kwale. $DH_1$5 and $DH_1$18 had their means lower than that of the two parents but

significantly lower than Kwale. There was no significant difference between the means of the two DHs produced from the Duma x Kenya Chiriku F_1. The $DH_1$11 had a mean number of 1.00 sterile spikelets per head lower than the two parents. $DH_1$12 with a mean of 2.0 sterile spikelets per head was similar to Duma but lower than Kenya Chiriku.

4.2.8 Number of grains per head

Nine Doubled Haploids; $DH_1$12, $DH_1$7, $DH_1$11, $DH_1$14, $DH_1$1, $DH_1$5, $DH_1$6, $DH_1$19 and $DH_1$18 were superior in grain yield to both parents. The $DH_1$13 and $DH_1$2 were higher in grain yield than the poor parent (Table 6).

A few DHs namely $DH_1$3, $DH_1$4 and $DH_1$17 had lower grain yields than both parents. These results are in line with Gland and Schmuetz, (1991) findings that DHs show a favourable combination of high grain yield, 1000 – grain weight and protein content. This is an indication that DHs can reach the heterosis of hybrid. The best DH ($DH_1$12) had a mean grain yield of 44 grains/head while $DH_1$17 had the lowest mean grain yield of 4.38 grains per head. Henry and de Buyser (1991) obtained similar variability in the yield of grains in wheat.

The ultimate goal of any breeding program in wheat is to increase yield. The yield is determined by the number of grains produced and their weight. The response of plant to drought at all the stages from germination to anthesis is reflected at grain filling stage. The ability of the plant to effect pollination even at low moisture condition is shown by sterility level and number of grains per head.

The entries were significantly different for grain yield. There was great variation between the entries. In case of Kenya Mbweha x Kwale derived DHs no significant difference was observed between their mean yield but all the DHs had more grains per head than both of the

parents. This is an indication of heterosis as observed by Henry and de Buyser (1991). The DHs derived from F_1 can be reach the heterosis of hybrids.

Similar results were obtained from the DHs of Kenya Mbweha x Kenya Chiriku ($DH_1$7 and $DH_1$14), which had mean grain number of 36.67. $DH_1$7 had a mean grain number of 43.49 as compared to the parents at 24.33 and 30.67 for Kenya Mbweha and Kenya Chiriku respectively. There was much variability between the parents and the DHs derived from Kenya Ngamia x Kwale but all the DHs had a mean grain number falling between the means of the two parents.

The $DH_1$2 and $DH_1$13 had a mean grain of 41.49 and 41.76 respectively, which was significantly higher than the mean of Kwale at 27.94 grains/head. $DH_1$3 and $DH_1$10 had mean grain number of 24.94 and 27.67 respectively, which were significantly lower than the better parent, Ngamia at a mean of 41.67. $DH_1$15 with a mean grain number of 30.00 was intermediate and was not significantly different from either of the parents.

$DH_1$17 and $DH_1$20 both produced from Ngamia x Kenya Chiriku F_1 had their mean grain number lower than their parents. The $DH_1$17 had a significantly lower number of grains than the parents. $DH_1$20 with a mean of 28.667 grains per head was significantly lower than Ngamia but not significantly different from Kenya Chiriku.

Only one of the five DHs produced from Duma x Kwale F1 had a mean grain number lower than both the parents although not significantly. The other DHs had their means falling above the means of the two parents. This is another indication of heterosis or additive gene effect. $DH_1$5 had the highest number of grains of 35.49 which was above the two parents, namely

Duma at 28.33 and Kwale at 27.94. Generally there was no significant difference in grain yields between the parents and the DHs.

$DH_1$11 and $DH_1$12 both produced from the Duma x Kenya Chiriku F_1 had higher yield than both parents. $DH_1$11 with a mean grain number of 40.94 was higher than that of Duma but not significantly. $DH_1$12 with a mean grain yield of 44.00 was significantly higher than Duma but although higher than Kenya Chiriku the difference was not significant.

For all the DHs studied in this experiment, it is clear that DHs were in general higher than one parent or in a few cases higher than both parents. This character has a direct relationship with drought tolerance. A plant can only be said to be drought tolerant if it is able to produce seeds. One of the fundamental contributing factors to yield in any situation is the inherent ability of a genotype to translate input into output. If a genotype is more efficient to utilize the scarce water to produce higher yields then it is more droughts tolerant (Henry and de Buyser, 1991). de Buyser *et al* (1981b, 1985) also indicated that the yield of DHs will depend on the parental cross and demonstrated that the agronomic value of DHs depends on the genetic potentiality of the parental cross and not on the technique of producing the DHs. de Buyser et al (1987) showed that DHs having significantly higher productivity or similar to the best variety can be obtained. From F_1 the yield differences between the best and the worst DH can reach 20 to 30 %, depending upon environmental conditions (de Buyser *et al* 1985).

4.2.9 Weight of grains (g)

The drought tolerant varieties (Kenya Mbeha, Ngamia and Duma) had their mean grain weight above the overall mean. $DH_1$12, $DH_1$10, $DH_1$4, $DH_1$6, $DH_1$18, $DH_1$2 and $DH_1$16 had

their mean grain weight above the overall mean (Table 6). The highest DH was $DH_1$12 with a grain weight of 0.42gms. This was higher than its parent Kenya Chiriku.

Kenya Chiriku and Kwale had a mean below the overall mean. The DHs with a mean above overall mean can be taken as tolerant to drought especially at grain filling stage. They were able to assimilate foods during this period hence higher weight. The grain weight was a good measure of transfer of yield into the drought tolerant cultivars from the high yield cultivars because DHs were superior even to the drought tolerant varieties.

Due to the low number of grains per entry, 10-kernel weight were taken.1000 grains weight had been shown by Henry and de Buyser (1991) to have high variability between the DHs and these DHs were distributed in both direction around the parents. This trend was reflected in our experiments (Table 6). There was significant difference between the DHs. The CV was 12.26, which was within the range of Agricultural experiments indicating that the mean weights were distributed round the mean. In most of the DHs the grain weight ranged between the two parents.

The normal distribution is clearly shown by the DHs produced from the Kenya Mbweha x Kwale F_1. $DH_1$1 had a mean kernel weight of 0.30g, which was lower than that of the two parents. $DH_1$19 with a mean kernel weight of 0.27 g was also lower than both parents and significantly so with Kenya Mbweha. $DH_1$8, which had a higher weight than Kwale but lower than Kenya Mbweha.

For the two DHs produced from Kenya Mbweha x Kenya Chiriku F_1 there was no significant difference between them and the parents although $DH_1$7 had a mean grain weight lower than

both the parents while $DH_1$14 had a mean grain weight of 0.343g which was better than that of Kenya Chiriku (0.312g) but lower than that of Kenya Mbweha (0.367g).

Of the six DHs produced from Ngamia x Kwale F_1, only $DH_1$3 had a mean grain weight significantly lower than both the parents. Other DHs such as $DH_1$13 and $DH_1$16 had their means lower than those of the two parents but not significantly. DH $_1$15 with a mean grain weight of 0.37g was between the means of the two parents. Only $DH_1$2 was able to combine high grain number and high grain weight in this group. $DH_1$17 and $DH_1$20 both produced from Ngamia x Kenya Chiriku F_1 had their mean grain weight lower than that of both parents significantly lower than that of both parents.

The parent Duma showed a significant superiority in grain weight over its DHs and other entries in general. Duma was significantly superior than all the DHs produced from the cross between it and Kwale. $DH_1$4 and $DH_1$9 with mean grain weights of 0.305g and 0.267g respectively, were lower than that of both the parents but significantly so with Duma. $DH_1$5, $DH_1$6 and $DH_1$18 with means of 0.310g, 0.367g and 0.326g, respectively fell between the two parents means but were significantly lower than Duma but not significantly higher than Kwale.

$DH_1$11 had a mean grain yield of 0.307 g, which was lower than the mean of the two parents. $DH_1$12 with a mean grain weight of 0.42g was significantly higher than Kenya Chiriku but not significantly lower than Duma.

The numbers of grains per head and grain weight are important components of grain yield (Machando *et al.,* 1993). In some DHs there is an indirectly correlation between grain number and weight. The fewer the grain number, the heavier they were and vice versa.. Some DHs

were able to combine high grain number and weight. These included $DH_1$8, $DH_1$2, $DH_1$6 and $DH_1$12. These DHs, which were produced from different combinations of parents, either were superior than the poor parent or were as good as the best parent.

$DH_1$12 had the best combination of the number of grains (44) and grain weight (0.42 g), which was as good as the best parent (Duma), (0.42g). $DH_1$7 with 43.49 number of grains was poor in grain weight. DHs with high grain number but poor grain weight indicate that they were poor in tolerating drought at grain filling level stage of growth. DHs with low grain number but high grain weight indicate that they were poor in drought tolerance at anthesis but good at grain filling stage.

4.3 Tolerance to Drought of the 20 DH Lines

The twenty DHs had a wide range of variation towards drought stress. In all the parameters measured, it was clear that each DH was able to tolerate drought at different stages of growth and in different ways. $DH_1$7, $DH_1$2, $DH_1$14 and $DH_1$12 were able to combine grain weight and grain number and were better in withstanding drought than both their parents. This was in line with the findings of both Ba Bong and Swaminathan (1995) and Henry and de Buyser (1991) who had showed that DHs show positive transgressions or heterosis. Although some of the DHs were lower in grain yield than the two parents only DH13, DH117 and DH120 had a significantly lower grain yield than the two parents. Similar results were obtained in wheat (Henry and de Buyser, 1991) where they reported that no DH are worse than their parents in terms of yield.

The yield difference between the highest and the lowest DH was 1.32 g per head. This was 70 %and was quite high compared to the work of de Buyser *et al.* (1985) who had a mean percent difference of 30%. This difference was mainly influenced by the wheat genotypes used to develop the DHs (de Buyser et al 1981b 1985, 1987). This difference can also be taken as an indication of a rich recombination for selection.

Although Wenzel (1995) had shown that at least 100 DH lines are required to obtain superior lines relative to yield we were able to obtain 4 superior lines from 20 DH lines. This was due to the fact that the success depend not only on the genes one is trying to combine but also on the choice of the parents (Kasha 1995). The genotypic effect on the efficiency of selection is clearly shown by $DH_1$117 and $DH_1$120 (Table 6) which were selected from Ngamia x Kenya Chiriku F_1. Both these DHs had a mean grain yield per head lower than that of the lower parent, while $DH_1$11 and $DH_1$12 derived from Duma x Kenya Chiriku F_1 were better than

their parents. This also shows an indication of the efficiency of the DH technique in the recombination of additive genes.

There was a general tendency of the performance of the DH lines being distributed in both directions around the performances of their parents. Of the 20 DHs, 14 were not significantly different from the parents in terms of yield . This was also reported in tobacco (Deaton *et al.*, 1982). Although only a few DHs showed heterosis there was no exhibition of reduction of vigour in all the DHs except $DH_1$17 and $DH_1$20. Such results were observed by Deaton et al (1982).

The DHs can be divided into four categories:- (i) superior or equal to parents in both grain number and grain weight, (ii) superior than parents in grain number but not in grain weight, (iii) superior in grain weight but poor in grain number and (iv) inferior in both grain number and weight (Bitch *et al* 1991).

The degree of drought tolerance of 20 DH lines is given below. The $DH_1$14 $DH_1$2 and $DH_1$12 were superior to their parents on both grain counts per head and grain weight. $DH_1$1, $DH_1$8 $DH_1$19 $DH_1$7 $DH_1$13 $DH_1$5, $DH_1$6, $DH_1$9, $DH_1$18 and $DH_1$11 had superior grain number per head but lower grain weight for either one or both parents. $DH_1$10 and $DH_1$15 were superior in grain weight but low in grain yield per head. $DH_1$3, $DH_1$16, $DH_1$17, $DH_1$20 and $DH_1$4 were poor than their parents both in grain number per head and 10-grain weight. The following Dhshad good qualitiesfor drought torelance as described below:-

$DH_1$14 had a mean seedling survival of 50, which was not significantly lower than the best parent. It was 38.33 cm at tillering and was not significantly different from the parents. It took 70 days to reach anthesis, an average for early maturing varieties (Kinyua, 1991). Spikelets per head were 17.33, which was not significantly higher than that of its parents.

Number of sterile spikelets was significantly lower than its Chiriku parents. The length of heads was 7.42, which was not significantly different from the best parent.

The $DH_1$2 derived from Ngamia x Kwale was significantly shorter at tillering (25.64cm) than its parents. It was late maturing (89 days to 50 % anthesis), good number of spikelets (19.1) which was significantly higher than that of the lower parent. Low sterility (1.01 spikelets per head) which was significantly lower than the lower parent and had head length of 6.17 cm length of head which was not significantly different from the parents. This DH showed superiority over its parents in most of the characteristics measured.

The $DH_1$12, a late maturing line (84.67 days) which was significantly later than its Duma parents (53.33) had good number of spikelets (17.33) and average sterility of 2 spikelets per head. A good size head of (8.53 cm) which was above the overall mean. This lines ($DH_1$12) showed superiority over their parents for most of the characters measured.

The $DH_1$1 produced from a cross between Kenya Mbweha x Kwale was superior in most of the characteristics compared to its parents except for grain weight. It had good grain yield although late maturing. $DH_1$8 another product of the crosses, Kenya Mbweha x Kwale was also good in grain yield and average in grain weight but late in maturity with 50 % anthesis at the 84th day. The lower grain weight of the two varieties was due to exposure to drought at grain filling stage (Hossain *et al* 1990).

The $DH_1$7 had higher grain yield (43.49 g/head) was superior to both parents and only second to $DH_1$12 but had low grain weight (0.28 g) which was lower than the overall mean. This was an early maturing line with average sterility and smaller size of the head. The $DH_1$13 had a mean grain yield (41.70 g) higher than both the parents and an average grain weight and a longer head. This indicates that this line can do better if enough rain is available at the

seedling stage (Kheiralla *et al* 1993). The low number of sterile spikelets indicates that this line has the ability to tolerate drought at booting, anthesis and grain filling stages.

$DH_1$11 another product of Duma x Kenya Chiriku had a high grain yield (40.94 g per plant) but had an average grain weight. It had an average head length (7.59 cm). It is an early maturing line indicating that it can do better if enough rainfall is available at seedling stage. The low yielding DH lines were significantly poor in most of the parameters measured as compared to their parents. $DH_1$3 with a mean grain weight of 24.94 g per plant was significantly lower than the best of it parents, Ngamia at 41.67 and also very poor in grain weight at 0.21g it was average in head length, sterility and spikelets per head, late maturing. It was poor in seedling survival and very short at tillering.

$DH_1$16 although lower in most characteristics had a low sterility rate which was significantly higher than the two parents this also compare well with $DH_1$4. $DH_1$17 was significantly inferior to both its parents and the other DHs. It had high spikelets sterility and had significantly lower seed weight than both parents. The critical stages were booting and anthesis which determined the size of the head and the number of sterile spikelets. The DHs that withstood drought at these stages were higher in grain number and grain weight.

$DH_1$12 with a low sterility and long head showed that it was very tolerant at booting and anthesis stage. The $DH_1$14 was another line although late maturing was able to withstand the drought and produce large number of seeds of average grain weight. These DHs should be tested under different field conditions to confirm their ability to resist drought at various levels of development.

4.4 Correlation Analysis

The general correlation analysis was done for all the parameters measured (Table 7). The grain yield was negatively correlated with sterile spikelets and kernel weight. The kernel weight was also negatively correlated to the number of effective tillers, sterile spikelets and days to 50% anthesis though not significant, indicating that the higher the number of effective tillers the lower the grain weight. The lack of significance in these relationships is an indication that the DHs could combine most of the characteristics resulting to an increase in yield.

The results indicated that heads with few seeds had high grain weight. This is because the fewer the number of developing seeds the more the assimilates were directed to each of them. Grain weight was positively correlated with germination percentage and grain number. Those lines that germinated well had more grains of reasonable weight. This was interesting to note that most of the DHs were able to combine high grain weight and reasonable number of grains, a true measure of drought tolerance.

A line, which has a high germination percentage, few tillers at booting, reasonably long head will most probably have many fertile spikelets. Such a line will have more grains of reasonably high weight. From such a description the following DHs may be categorized as drought and high yielding; $DH_1$2, $DH_1$7, $DH_1$12 and $DH_1$14.

Table 7. Correlation coefficient for different parameters in DH lines.

	GP	TB	ET	LH	SP	GR	SPT	DA	GWT
GP	1								
TB	-0.035	1							
ET	0.102	0.27**	1						
LH	0.05	0.09	0.05	1					
SP	0.078	-0.0114	0.33**	0.108	1				
GR	0.066	0.142	0.051	0.031	0.066	1			
SPT	-0.0196	0.022	-0.222	0.033	-0.176	-0.29**	1		
DA	-0.162	0.0192	0.041	0.207	0.169	0.145	0.094	1	
GWT	0.316	0.116	-0.055	0.28**	0.05	0.28**	-0.25**	-0.136	1

** Significantly correlated at $P \leq 0.05$

GP – Germination percentage, TP – Tillers at booting, ET – Effective tillers, LH – Length of head, SP – Spikelets per head, GR – Grains per head, SPT – Sterile spikelets per head, DA – Days to 50% anthesis, GWT – Weight of ten kernels.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. Successful crosses were made between the three drought tolerant varieties namely Ngamia Kenya Mbweha, and Duma and the two high yielding varieties namely Kenya Chiriku and Kwale. The F_1s were grown *in vitro* to enhance maturity.

2. The haploids from each of the F_1s of different crosses were developed by crossing them with maize pollen. Although there was some variation in response to haploid development in various F_1s the technique was highly successful and there was no genotypic differences. On average at least 3 embryos per head were produced using this technique.

3. Doubled haploids were developed from haploids successfully by colchicine treatment. The number of DHs developed from haploids varied from one cross to another but this variation was attributed more to environmental factors rather than the genotypic factors.

4. At least four DHs performed better under drought conditions than their parents in terms of grain number and grain weight indicating that this component of yield were recombined in the drought tolerant DH lines.

5. For the self-pollinated plants like wheat, where plants are tested after attaining homozygosity DH technique was found superior to the traditional breeding methods in saving time from seven years to two years.

5.2 Recommendations

1. More tests should be carried out on the DH lines especially for yield under optimum field conditions to verify their potentiality and stability in drought prone areas.
2. When using the DHs technique it is suggested to confirm DH_1 for homozygosity in DH_2s and advanced generations before they are tested for various agronomic characteristics.
3. The protocol on haploid production should be improved especially at the embryo rescue stage to increase the number of haploids produced per head.
4. More research should be done to verify whether: (I) Maize has genotypic effect in response to haploid production, (ii)Which is the best maize variety to be used for the hybridisation process and, (iii)For how long can maize pollen be stored and still maintain viability.
5. More studies are required to show whether the maize genome has an influence in the development of the wheat haploids, i.e. is there gametoclonal variation when the wide hybridisation is used and if there is, is it of any agronomic importance?

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APPENDICES

1.0 The doubled haploid production protocol

The following protocol can be used in the production of haploid and doubled haploids in wheat using maize. In wide hybridisation, as in this protocol, the environmental conditions should be taken seriously for their play an important role in determining the success of haploid production , plantlets regeneration, cholchicine treatment and production of doubled haploids.

1. F_1 production and development.

   The plants to be used for the F_1 production are grown in the cage. Synchronization should be done to make sure that anthesis comes at the same time for the both parents. Emasculation and pollination of the female parent is done. The F_1 seeds are harvested in 18 days after pollination and the embryos excised under sterilized condition. The embryos are incubated on to an agar culture medium without hormones. The plants are then transferred to the potted soil in the green house. The plants should be kept at 25 ° c and 16 day length. At booting the plant should not suffer from water stress

2. The media

   MS-medium half strength enriched with 0.8 % Agarose and 2 % sucrose and vitamins should be used.

3. Embryo rescue.

   Plants are emasculated two days before anthesis and pollinated with fleshly collected maize pollen. The culm of the emasculated head is then injected with 100 ppm 2,4-dichlorophenoxyacetic acid (2,4–D) two days after pollination. This is repeated two to three times. The temperature should be maintained at 25°c and the humidity should be saturated. Polythene bags should be used to increase the moisture around the head. At this time temperature and moisture are very important in the success of haploid production.

The caryopsis are harvested 15 days after pollination. They are sterilized using 2 % sodium hypochlorate for 15 minutes and washed with sterilised distilled water. The caryopsis are dissected and the embryos which are about 1.5 mm removed and placed in the medium.

4. Embryo culture.

The embryos are placed in a culture cabinet at 25° C under 5000 lux Light. They are then placed in the growth room with 16 hour day length and at 25 °C temperature under Ca 5000 lx. Light intensity. The cultured plants with fully developed roots and leaves are transplanted from the agar medium in potted soil. This should be in moist room at 22° C.

5. Chromosome doubling

At tillering stage the roots are cut to about 2 cm below the crown. The leaves are trimmed then immersed in 0.2 % colchicine, 2 % DMSO and 15 drops of mean 20 solution for 5 hours at 20°. Wilting of the plants before treatment increases the colchicine effect.

The plantlets are then washed with tap water and replanted in potted soil. The florets that set seeds always have normal anthers, compared to the shriveled ones of sterile florets.

2.0 Schedule of haploid and doubled haploid production through the Wheat x Maize technique in Wheat.

Week	Procedure	Efficiency/ weekly per person
0	The emasculation and pollination of wheat florets with maize pollen	500 florets
2	Excise immature embryo from crossed seeds and culture invert	350 embryo
6	Transfer regenerated plantlets from culture medium to soil	350 plantlets
10	Treatment of haploid plants with Colchine solution	180 haploids
26- 30	Harvest seeds from the DHs	150 DHs
46-60	Planting and evaluating the DHs for drought tolerance or other agronomic stress.	100 DHs

From the above schedule then it is possible to produce and analyse the DHs within a period of 1 ½ years.