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Germination response of cat's whiskers (*Cleome gynandra* L.) seeds to heat shock, potassium nitrate and puncturing

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The effects of subjecting landrace (ZV) and a cross (CV) of cat's whiskers, *Cleome gynandra* L. seeds to heat shock at 40ºC for 5 days in an incubator and placing them in an incubator and germination room; soaking in 0, 2% Potassium Nitrate and pre-chilling them at 4ºC for 7 days before placing in incubator and germination room, and puncturing the seed coat with a needle were evaluated in 2011 at the Tobacco Research Board in Zimbabwe. Germination response was measured 4, 8 and 12 days after sowing. Growth environment significantly (p=0.74) affected germination. Seeds germinated in the germination growth room had significantly higher germination percentages compared to those germinated in the incubator. The puncture test showed the highest germination percentage in the first four days. Germination percentage 8 days after sowing was not significantly different from the germination percentage after 4 days. Twelve days after sowing germination percentage still did not show a significant improvement. The heat shock treatment of ZV showed the lowest germination percentage 12 days after sowing showing an even lower germination percentage compared to the control of the same variety. It is recommended that nurseries, which have the capacity to break *C. gynandra* dormancy by using chemicals for scarification or performing the puncture test can produce seedlings which can be made available to farmers throughout the year. Puncturing the seed coat using a needle under a microscope is tedious and impracticable to the smallholder farmer. Therefore to match the propagation and cultivation with increased consumption of *C. gynandra*, there is need for further research to come up with pre-sowing treatments to solve the problem of dormancy.

Key words: *Cleome gynandra*, germination response, heat shock, potassium nitrate, puncture test

Introduction

Indigenous vegetables have played a crucial role in food security and in improving the nutritional status of the marginalized communities. Indigenous vegetables in tropical Africa have been reported to have medicinal properties

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which include anti-bacterial, anti-rheumatic, anti-cancer and anti-malarial activity (Chipupura *et al.,* 2011). Despite these important attributes, traditional vegetables have been neglected as they are associated with the poor rural lifestyle, hence are regarded as low status foodstuffs (Fusire, 2008). Cat's whiskers, *Cleome gynandra* (*Cleome*) (Capparaceae family) is one of the popular indigenous vegetables found in Zimbabwe (Ngwerume, 1997). It is an erect glandular-pubescent annual herb (Anbazhagi *et al.,* 2009). *Cleome* belongs to the botanical family Capparaceae, subfamily Cleomoideae and is related to *Cleome hirta* and *Cleome monophylla*. The genus is a phylogenetic of the Cruciferae (Brassicaceae) and the family contains about 700 – 800 species of which 50 occur in Africa (Chweya and Mnzava, 1997). The plant shows all the characteristics needed for efficient C_4 photosynthesis, including alterations to leaf biochemistry, cell biology and development, and belongs to the NAD-dependent malic enzyme subtype (Marshall *et al.,* 2007). This adaptation mechanism enables it to survive in drier and hot environments (Chweya and Mnzava, 1997; Schippers, 2000; Muasya *et al.,* 2009; K'Opondo, 2011).

The vegetable is often sold fresh or dried both in the rural and urban areas during the rainy and dry seasons. The plant, which is believed to be indigenous to Africa, is grown as an ornamental flower in the USA. *Cleome* contains vitamin A, B, C, iron, calcium, magnesium, antioxidants and micronutrients recommended to supplement medicinal therapy in fighting certain diseases like HIV and AIDS (Grubben and Denton, 2004). *Cleome* is the only vegetable whose Vitamin C does not drop in much water. It has high iron content (Schönfeldt and Pretorius, 2011) and Vitamin A. For expecting or nursing mothers and children below 5, deficiency in vitamin A leads to irreversible sight problems. Iron assists in brain and spinal cord development. Adequate supply of iron improves the reasoning capacity of children and helps them perform better in school. It is recommended that expecting and nursing mothers take in sufficient amounts of iron to prepare children to be bright. *Cleome* provides a cheap and rich supply of iron. The roots are chewed to induce uterine contractions and removal of retained placenta and control post-partum bleeding in childbirth process. The roots, leaves and flowers are used in the prevention of miscarriages and treatment of colic pains when boiled or cooked as food (Kamatenesi-Mugisha *et al.,* 2005). The leaves, roots and flowers are chewed, cooked or are sun-dried and drank in tea to treat sexual impotence or erectile dysfunctions in men (Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha and Oryem-Origa, 2005).

Propagation of *Cleome* is mainly by seed. Low germination capacity has been observed in *Cleome*. One reason for the poor germination of *Cleome* has

been attributed to the differences in times of male and female flower production. If female flowers develop earlier than the male flowers, by the time the male part matures the female flowers will have already dropped off and thus no pollination will take place. The same applies if male flowers develop earlier than the female flowers. It is only the mature seed that goes into dormancy, yet at harvesting the farmer may take the whole plant and collect all seeds, when the seeds were at different maturity stages. Increasing seed rates at planting will improve crop stands.

Dormancy is defined as the mechanism which makes viable seeds fail to germinate when exposed to favourable conditions for germination (Mashingaidze, 2000). It is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage and Leubner-Metzger, 2006). Dormant seeds do not germinate when imbibed in water even when conditions are favourable for germination. The hydrated seeds remain viable, but growth-arrested for weeks due to unknown restrictions within the embryo (Goldmark *et al*., 1992). Events associated with dormancy release during seed germination still require explanation. Seeds of *Cleome* are characterised by low germination and there has been no explanation for this (Ochuodho, *et al.*, 2005). Stuppy and co-workers (2010) suggest that seeds of *Cleome* may have physiological dormancy which needs to be overcome by mimicking the seasonal patterns of the species' native habitat. Physiologically mature seeds are either dormant or non-dormant and will germinate once the dormancy is relieved or optimum conditions are provided (Ochuodho and Modi, 2007). Early seed harvest may be desirable for seeds of some crop species. Fruits of cat's whiskers are dehiscent and must be picked as soon as they are ripe but before the fruits dehisce to release the dry seeds that drop to the ground (K'Opondo, 2011). The three types of dormancy are innate dormancy, induced dormancy and enforced dormancy.

Innate or primary dormancy is caused by conditions within the seed that prevent germination even if the seed is exposed to favourable conditions. It is inherited by the seed when it is released from the parent and it is caused by an immature embryo, impermeable seed coats which do not allow gas to enter, or could be due to genetically controlled imbalances in growth substances within the embryo. Induced or secondary dormancy is a result of conditions to which the seed or vegetative part is exposed after release from the parent. The seed may germinate when shed from the plant by 'acquired dormancy' when exposed to specific conditions like high temperatures and high carbon dioxide concentrations which induce the seed to go into a dormant state (Mashingaidze, 2000). Enforced dormancy occurs when the seed fails to germinate due to

unfavourable environmental conditions such as shortage of water, low temperature and poor aeration.

It has been argued that there may be no need to invest in chemicals to overcome *Cleome* seed dormancy as simply soaking seeds in water is thought to break *Cleome* dormancy (Ojiewo, pers. com.[1]). Research carried out by Ekpong (2008) in Thailand, showed that *Cleome* seed needed to be subjected to various pre-sowing treatments which included soaking the seeds in various levels of giberrellic acid (GA), potassium nitrate, pre-chilling, soaking and pre-heating the seed to break dormancy. Brown (2002) subjected *Cleome* seeds to cycles of wetting and drying to remove germination inhibitors and improve water uptake when seeds are planted. Fusire (2008) observed that seed dormancy of *Cleome* could be broken by seed priming which included dipping the seed in hot water for 30 seconds and then immediately in cold water. It has been suggested that rapid heating and cooling can break the seed coat. Exposing the seeds to direct sunlight for at least five hours before planting and alternatively adding wood ash in the planting stations before placing the seed are other practices that are thought to remove dormancy and improve germination percentage. Ochuodho and company (2008) subjected *Cleome* to alternating temperatures and found that seeds incubated at 20ºC had low germination percentage. It was also noted that seed germination proceeded slowly at temperatures below 21ºC.

Materials and methods

The research was carried out in a laboratory at Kutsaga Research Station in Harare. A cross of an improved variety of *Cleome* from Kenya and a landrace from Zimbabwe (designated CV) which was obtained from a Marondera *Cleome* researcher; and a landrace from Zimbabwe (designated ZV) which was obtained from the Community Technology Development Trust were used in the experiments. A digital scale was used to weigh 1g of seeds (1 250 seeds) from each of the seed lots. Seeds were placed in Petri dishes and sprinkled with distilled water. The two Petri dishes were placed in an incubator to subject the seeds to heat shock at 40ºC for five days. Two more Petri dishes containing 1g of seeds from each of the seed lots were placed in the Petri dishes lined with cotton wool and filter paper. The seeds were imbibed in 0.2% potassium Nitrate (KNO^3) and pre-chilled in a refrigerator at 4^oC for five days. 1 gram of ZV seeds was also placed in a Petri dish and the seeds sprinkled with distilled water to imbibe for 5 days.

The seeds subjected to heat shock were placed in glass Petri dishes lined with wet cotton wool and Whatman filter papers moistened with distilled water

for the duration of the experiment. The experimental design was a two factorial completely randomized design (CRD) with three replications each.

Three of each of the seed lots in a Petri dish were placed in a germination room with a temperature of 25±3ºC and the other three Petri dishes were placed in the incubator with controlled temperatures maintained at 20-30ºC. Similarly, the seeds imbibed in KNO^3 and subjected to heat shock were put in six Petri dishes per seed lot and they were placed three each in the germination room and three each in the incubator. ZV seeds imbibed in distilled water were punctured with a needle under a microscope and also placed in six Petri dishes. Three of the Petri dishes were placed in the germination room and the other three in the incubator. CV seeds were not subjected to a puncture test as the seeds in a pre-trial experiment had shown 100% germination. Untreated seeds from both seed lots were also placed in six Petri dishes: three in the germination room and the other three in the incubator and these served as the controls. Germination was recorded daily for 12 days and germinated seeds were removed from the Petri dish. The criterion for germination was a visible radicle emergence.

Data analysis

Data were analyzed using GenStat Statistical package. The percentage data ranged from 0 to 100 and were therefore arc sine transformed (Gomez and Gomez, 1984).

Results and discussion

Germination of *Cleome* was significantly affected by growth environment ($p=0.74$). Seeds germinated in the germination growth room had significantly higher germination percentages compared to those germinated in the incubator. Results from the experiments are presented below.

Growth environment

Results on effects of the growth environment on germination of *C. gynandra* was illustrated in Fig.1.

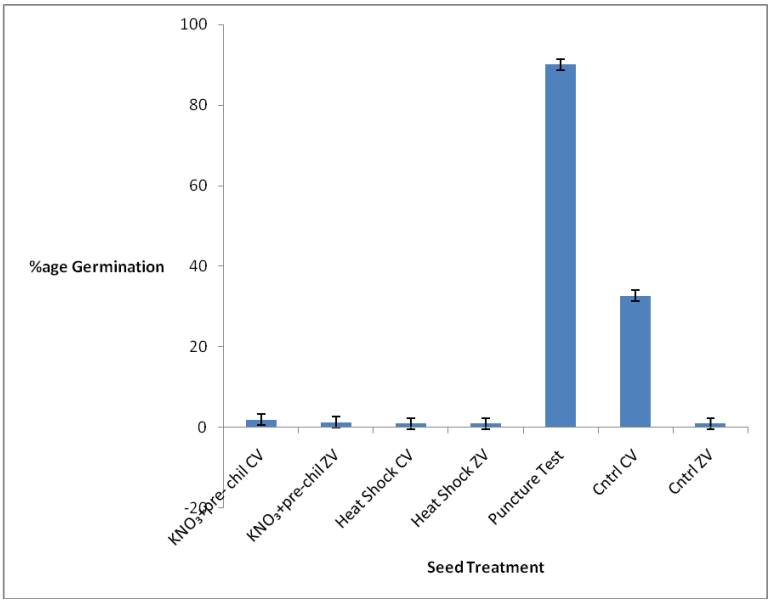

Fig. 1 Percentage germination of *Cleome* 4 days after sowing

Percentage germination of the puncture test and the control for the cross variety were significantly (p=0,74) different from the potassium nitrate and pre-chill of both varieties, heat shock of both varieties and the ZV control four days after sowing. There was no significant difference in the germination percentage of *Cleome* on the other five treatments (p=0,001). The puncture test showed the highest germination percentage in the first 4 days. This is in contrast to Ekpong (2008)'s experiment in which he noted that pre-heating *Cleome* at 40ºC for 5 days was the most effective method of breaking dormancy. Variations due to the different treatments used on the two seed lots suggested that the magnitude of differences in varieties had a significant effect on germination of Cleome. The different growth conditions also suggested the conditions suitable for improved germination in *Cleome.*

Stuppy and fellow researchers (2010) noted that *Cleome* seeds were difficult to grow because the species had physiological dormancy and this type of seed dormancy can be overcome by mimicking the seasonal patterns of the species' native habitat of optimum temperatures. The high temperatures in the growth room and the constant temperatures which did not fluctuate a lot could have contributed to the high percentage germination compared to the incubator whose temperatures fluctuated by 10ºC during the night. Fluctuations in temperatures affect germination (Ochuodho *et al.*, 2008). Seed germination proceeds slowly at low temperatures compared to higher temperatures (Klimaszewska *et al.*, 2004).

Cleome seed responses to duration of exposure on germination are summarized in Figure 2.

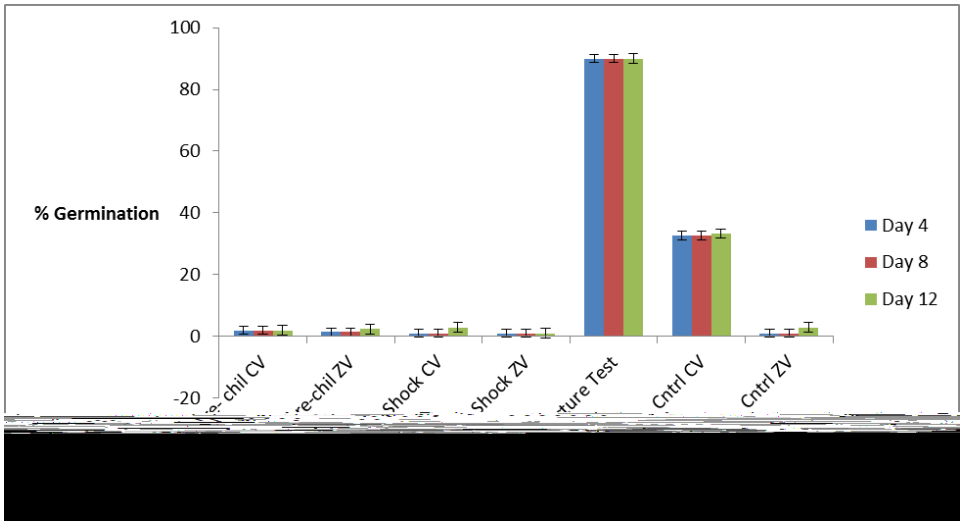

Fig. 2 Effects of duration of exposure of *C. gynandra* on germination

There was no significant increase in germination percentage from Day 4 to Day 12. The ZV control showed a germination percentage slightly higher than the control on the twelfth day and this was probably because the heat shock might have affected the embryo of the seed thereby affecting germination. The percentage germination was not significantly different when germination counts were made 4, 8 and 12 days after sowing. Ochuodho and co-workers (2008) assessed the photoinhibition influences and protein utilization during seed germination in *C. gynandra.* They discovered that germination was negatively affected by the number of days after sowing because after breaking dormancy, protein bands of prominent protein complex 28 – 32 kDa and 18 – 20 kDa observed in mature seeds of *Cleome* disappeared rapidly during germination.

Conclusion

Of the three methods that were evaluated in this study, mechanical scarification by puncturing the seed coat with a needle is the most effective method of breaking dormancy in *Cleome.* It is recommended that nurseries, which have the capacity to break dormancy by using chemicals for scarification or perform the puncture test, can produce seedlings which can be made available to farmers throughout the year. However, puncturing the seed coat using a needle under a microscope is tedious and impracticable to the