Overcoming Seed Dormancy in *Cleome gynandra* L. to Improve Germination

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ABSTRACT

Cleome gynandra L. is a traditional semi-domesticated leafy vegetable grown in East Africa. It belongs to the family *Cleomaceae*. Farmers save seed from each harvest for planting in the next season. *C. gynandra* seeds fail to germinate when planted immediately after harvest. Studies were conducted to determine appropriate seed treatments to overcome dormancy in freshly harvested *C. gynandra* seeds. Seeds were subjected to various dormancy breaking treatments including: potassium nitrate (KNO_3), leaching, light, gibberellic acid (GA_3) and chilling. Additional studies were carried out to determine the degree of dormancy in seeds harvested from pods at different positions on the plant. Among all treatments studied, application of GA_3 at a concentration of 500 ppm resulted in the highest final germination. Stratification for two weeks at 5 °C and germination in dark also improved germination significantly. While leaching had no significant effect, treatment with KNO_3 and light reduced germination. Seeds from lower and middle positioned siliques germinated readily compared to those from upper siliques. We suggest for commercialization of *C. gynandra* seed that have high potential germination, treating seeds immediately after harvest with GA_3 at a concentration of 500 ppm before being packaged and sold to farmers is a possibility that can be explored.

INTRODUCTION

Cleome gynandra L. (syn. *Gynadropsis gynandra* (L.) Briquet and *C. pentaphylla* L.; Edmonds and Chweya, 1997) is an indigenous, semi-domesticated leafy vegetable grown throughout East Africa (Chweya, 1997) and is commonly known as spider flower, cats' whiskers and bastard mustard (Maundu et al., 1999). The genus *Cleome* belongs to the family *Cleomaceae* Bercht. & Presl. *C. gynandra* is a nutritious leafy vegetable rich in iron, magnesium, calcium, vitamins A and C, proteins, carbohydrates, phosphorous and fiber which are higher than in exotic vegetables (Arnold et al., 1985). It also has the medicinal use of restoring blood supply so women regularly consume it before and after giving birth and boys are fed the vegetable just after circumcision (Opole et al., 1995). The indigenous leafy vegetable plays a key role in diets of families in rural areas (Schippers, 2000; Jansen van Rensburg et al., 2004) and several farmer's landraces of *C. gynandra* are known but have not been classified. The plant is an erect herb that grows up to

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1.5 m tall with white, pink or lilac petals. Seeds are born in siliques that are green but turn yellow when ripe, and dehisce easily when dried to release the seeds.

Most indigenous leafy vegetables are not part of the formal seed sector and some species regenerate as weeds in cultivated fields while others are more difficult to propagate (Maundu et al., 1993; Simiyu et al., 2003). Like many other traditional leafy vegetables, *C. gynandra* is cultivated mainly by subsistence farmers. It is semi-domesticated in that farmers collect seeds from volunteer plants and propagate them for home consumption and, in some cases, for sale in local markets (Chweya and Eyzaguirre, 1999).

Sources of seed for planting include farm-saved, local markets, borrowed from neighbors and relatives, or collected from wild plants (Maundu et al., 1993; Schippers, 2000; Simiyu et al., 2003). The national seed company in Kenya, Kenya Seed Company, has attempted to produce indigenous vegetable seeds, including *Cleome* in small quantities, but they do so with limited information on their seed technology aspects (Ochuodho, 2005). Farmers identify the lack of good quality seed as a major constraint to optimal plant stands (Abukutsa-Onyango, 2007). *C. gynandra* seeds usually show low germination percentage in the field. Germination percentages under field conditions as low as 37 and 46% have been reported (Almekinders and Louwaars, 2000; Ndinya, 2003). The exact basis for the low germination is not clear but recent reports indicate that *C. gynandra* seeds exhibit considerable dormancy (Simiyu et al., 2003; Kamotho, 2004). This forces farmers to plant more seeds than necessary to obtain optimal plant stands.

Kamotho (2004) reported that dormancy in freshly harvested seeds of *C. gynandra* is broken by storage for six months to one year and the success of breaking dormancy depends on seed storage conditions. However, a long storage period for *Cleome* is not a common practice and farmers often use freshly harvested seeds or seeds stored for very short periods, usually less than three months. Thus, there is need to develop a dormancy breaking treatment that allows farmers to use fresh high quality seeds for growing the next crop. Attempts to meet this need had been previously made, but a study by Börhinger et al. (1999) investigating effects of light and temperature on germination of *C. gynandra* seed did not produce conclusive results. More recent experiments conducted on the effects of temperature, light and pre-germination treatments on germination of one to two year old *Cleome* seeds revealed that the highest germination percentage was achieved when alternating temperatures of 20–30 °C in the dark and scarification by puncturing the seeds at the radicle end improved germination (Ochuodho, 2005; Ochuodho and Modi, 2005; 2007). Since farmers plant freshly harvested seed which has been shown to have poor germination (Kamotho, 2004), the present study was conducted with the aim of assessing dormancy of seeds harvested from pods at different parts of the plant and to identify appropriate methods of reducing dormancy in fresh *C. gynandra* seeds to improve the production of this important leafy vegetable.

MATERIALS AND METHODS

The studies were conducted at Moi University, Chepkoilel Campus in Eldoret, Kenya. The location is within the Uasin Gishu plateau at Latitude 00°30′ N,

Longitude 35°15′ E and an altitude of 2180 m above sea level. Seeds from uncharacterized landraces were obtained from farmers in the western region of Kenya and were planted in a plastic house at the Department of Seed, Crop and Horticultural Sciences. Plants were grown under controlled environmental conditions to obtain uniform, high quality seeds for the germination experiments. Seeds used for the dormancy breaking experiments were harvested at maturity when the siliques were yellow in color. Seeds were dried to a moisture content of 5% (Kamotho, 2004) and used immediately. Seeds for prechill and leaching experiments were kept in a deep freezer at −20 °C till the time of the experiments. Seeds were subjected to existing dormancy breaking treatments as described below and incubated in a germination chamber set at temperature range of 20–25 °C, with relative humidity maintained near saturation. Untreated seed germinated with distilled water served as controls in all treatments. Apart from the light experiments where light regimes varied, all other experiments were conducted under 8 h of light and 16 h darkness. Germination counts were taken each day following planting and the last count was made on the fourth day except for light experiments where the last count was made on the fifth day. Protrusion of a radicle from the seed (radicle emergence) was considered germination.

Potassium nitrate (KNO_3)

A solution of 0.2% KNO_3 (ISTA, 2004) was used to moisten the substrate on which the seeds were germinated. A sample of 150 seeds divided into three replicates of 50 seeds each was planted on moistened filter paper in a covered Petri dish to avoid drying. Subsequent watering was done using distilled water.

Leaching

Leaching was conducted for a period of three days by completely immersing the seeds in distilled water, prior to testing for germination. Two samples of 150 seeds each in three replicates were used. In the first sample, water was changed daily while in the other seeds were held continuously without changing the water. The seeds were then placed in germination trays on filter paper wetted with distilled water and covered to avoid drying. A control set of seeds was planted and germinated alongside the leached seeds.

Light

Germinating seeds were exposed to cool white fluorescent light (approximately 1000 lux; ISTA, 2004) for daily photoperiod durations of: 24, 12, 8, or 0 h. Darkness (0 h light) was achieved by placing the germination trays in opaque black polyethylene bags. However, darkness was interrupted for less than 10 min each day for counting.

Gibberellic acid (GA_3)

GA_3 solutions of 200, 500 and 1000 ppm were used to moisten the filter paper on which the seeds were germinated (ISTA, 2004). A sample of 150 seeds divided into three replicates of 50 seeds was taken for each treatment.

Chilling (Cold stratification)

A sample of 150 seeds moistened with water was wrapped in aluminum foil and placed at 5 °C for 14 d (ISTA, 2004). The seeds were arranged in three replicates of 50 seeds each then germinated on moistened filter paper in petri dishes. A control set of seeds was planted and germinated along side the chilled seeds for comparison.

Silique position

Seed dormancy was assessed between seeds harvested from siliques at different positions on the plant. Fifteen *C. gynandra* plants were grown in a greenhouse in a wooden box (30 cm × 40 cm). At maturity (yellow silique), the plants were harvested at the base. Siliques were classified as lower, middle or upper according to their position on the plant by dividing the part of the stem producing siliques into three equal segments. Seeds were harvested separately for each position and bulked to provide the three seed lots used for testing germination. Each seed lot was divided into two groups. In the first group, 150 seeds in replications of 50 seeds each were germinated on moistened filter paper and incubated in the germination chamber at 20–25 °C, under 8 h of light and 16 h of darkness. The second group was germinated under 24 h dark except for the occasional few minutes exposure to light when taking data. Germination percentages were recorded from day one to day four.

Statistical analysis

The experiments were arranged in a Completely Randomized Design (CRD). Results were analyzed using descriptive statistics and analysis of variance (ANOVA) using Statistical Package for Social Scientists (SPSS) 10.1. Error bars for each data point were created using Microsoft Excel for the comparison of various readings within a treatment.

RESULTS

While seeds germinated with distilled water (control) had a germination of 56%, differences were observed in the final germination obtained with different dormancy breaking treatments. Seeds leached by immersing in distilled water either by changing water daily or without changing water showed a significantly lower germination percentage than the control at 2 and 3 d ($P < 0.05$) (data not shown). Seeds treated with GA_3 at a concentration of 500 ppm generally had significantly higher germination percentages than all the other treatments (Fig. 1). Chilling (cold stratification) was effective in breaking seed dormancy as the treatment had a final germination percentage significantly higher than the control (Fig. 1). Seeds germinated in darkness with brief intermittent exposure to light during counting each day had a significantly ($P < 0.05$) higher germination percentage than germination in distilled water (control). Treatment of seeds with KNO_3 significantly lowered the germination percentage below the control.

In the light experiment, final germination varied depending on the duration of exposure. In general, germination was suppressed with increased exposure to light. Thus, seed germinated in dark (with brief intermittent exposures to light during counting each day) had the highest germination (70%). Light

FIGURE 1. Cumulative germination of *Cleome gynandra* seeds under selected dormancy breaking treatments. Bars represent standard error ($P = 0.05$) values for the comparison of germination under different dormancy breaking methods and germination counts.

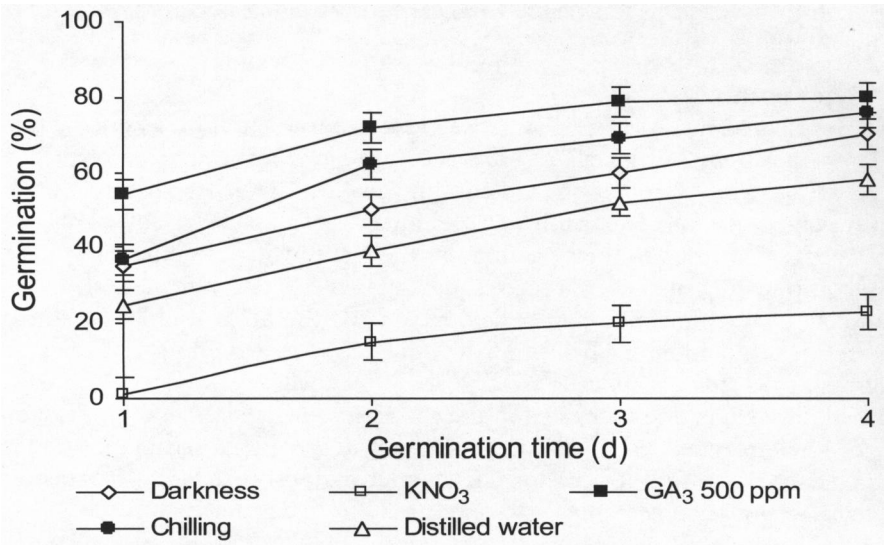
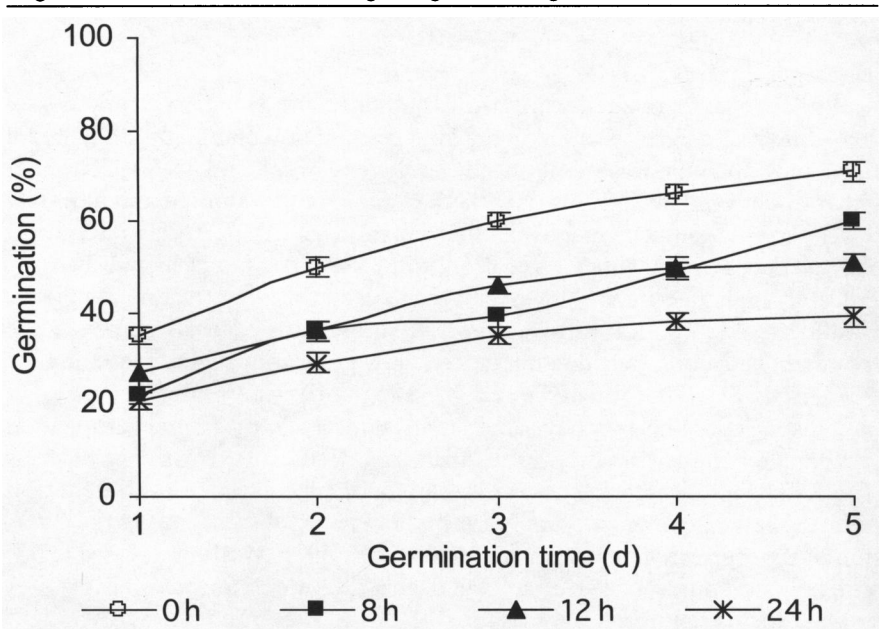


FIGURE 2. The effect of light in hours of exposure on germination of *Cleome gynandra* seeds. Bars represent standard error ($P = 0.05$) values for the comparison of germination under different light regimes and germination counts.



treatments of 8, 12 and 24 h, had final germination percentages 61%, 58%, and 38%, respectively (Fig. 2). Differences in germination between 8 and 12 h light treatments were not significant ($P < 0.05$) but were superior to germination at 24 h light treatment.

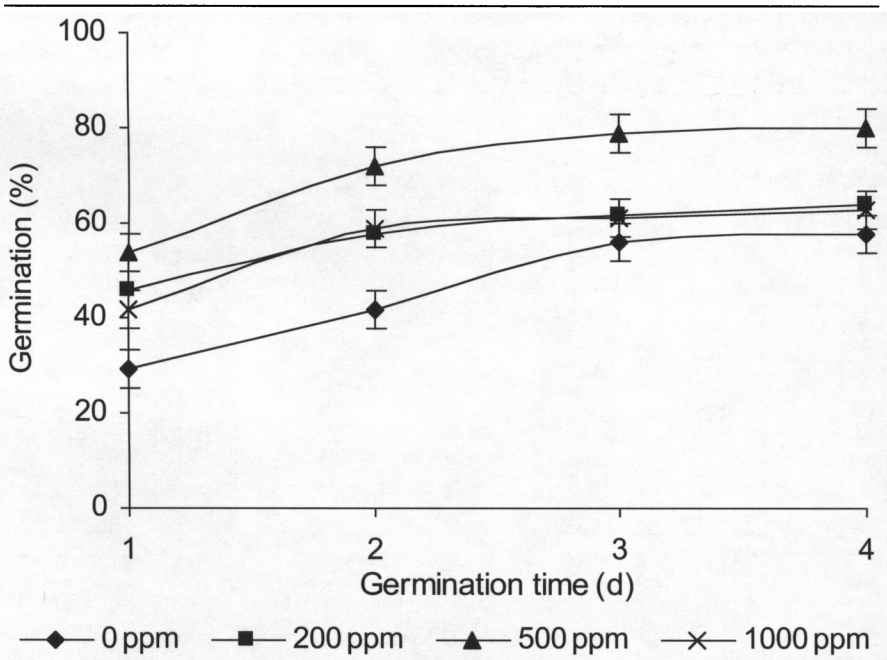
GA_3 concentrations of 200, 500, and 1000 ppm improved germination to 64%, 80%, and 63%, respectively. However, differences in final germination between seeds treated with 200 and 1000 ppm were not significant ($P < 0.05$) (Fig. 3). GA_3 treatment at 500 ppm was significantly superior in terms of improving *Cleome* seed germination.

Seed germination was affected by the position of the siliques on the parent plant. The seeds from the middle and lower positioned siliques showed significantly higher germination percentages than for the seeds collected from the upper siliques. This observation was noted for seeds germinated in both the 8 h light and dark regimes (Fig.4). However, within each light regime, differences in germination percentage between seeds from the lower and middle positioned siliques were not significant ($P < 0.05$).

DISCUSSION

Light has been reported to be an important factor for releasing seeds from dormancy in many plant species (Hilhorst and Karssen, 1988; Baskin and

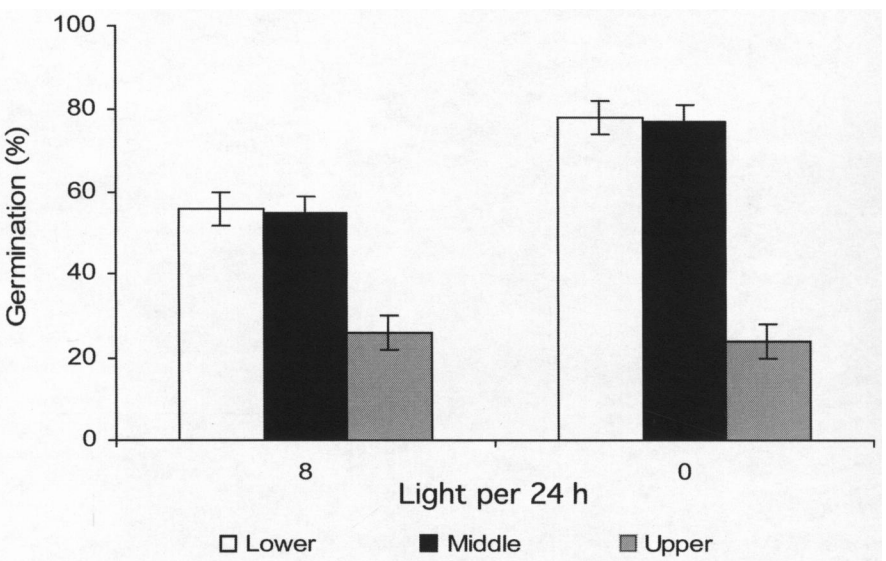
FIGURE 3. Dormancy breaking of *Cleome gynandra* seeds treated with different GA_3 levels, incubated in 8 h light and 16 h darkness daily. Bars represent standard error ($P = 0.05$) values for the comparison of different GA_3 levels and germination counts.



Baskin, 1998; Yamaguchi and Kamiya, 2002), but there are some species whose seed germination is enhanced in darkness and such species are said to be negatively photoblastic (Bewley and Black, 1994, Baskin and Baskin, 1998; Ochuodho and Modi, 2005). In the present study, *C. gynandra* seed germination was suppressed by exposure to light (Fig. 2). These results agree with reports by Ochuodho and Modi (2005; 2007) that exposure of *C. gynandra* seeds to light for longer than 12 h per day drastically reduced seed germination due to photoinhibition. Seed germination suppression by light is a commonly reported phenomenon for species which are physically dormant and produce small seeds (Bell et al., 1995). It is likely that light inhibition is a secondary 'protective' mechanism that ensures seeds that have lost physical dormancy do not germinate at the soil surface, where they may be prone to desiccation.

Potassium nitrate breaks dormancy by increasing the sensitivity of the seed to light (Goldmark et al., 1992). However in this study, KNO_3 did not enhance germination, an observation also recorded by Ochuodho and Modi (2005). *C. gynandra* seeds are negatively photoblastic and increasing their sensitivity to light using KNO_3 was not advantageous. Prechilling seeds at low temperatures (5 °C) for two weeks resulted in significantly higher germination than in the control seeds. Seed dormancy has been demonstrated to be relieved by imbibition at low temperatures (cold stratification) (Benvenuti et al., 2001). Chilling retards metabolic processes that act to inhibit germination in seeds then the germination process continues. It also breaks dormancy by production of GA. During stratification, changes in seeds include increased acidity,

FIGURE 4. Germination of *Cleome gynandra* seeds from pods located at the lower, middle and upper positions on the plant. The bars represent standard errors (P = 0.05) for the comparison of germination regimes of 8 and 0 h light per day.



water holding capacity, catalase activity, reducing sugars, respiratory rate, and vigor of the embryo (Bewley and Black, 1994).

Dormancy in freshly harvested *C. gynandra* seeds can be overcome by application of GA_3 at 500 ppm to the germination substrate. GA_3 breaks dormancy by inhibiting the action of abscisic acid (ABA), triggers the action of alpha-amylase that is a pre-curser for germination (Bewley, 1997), and promotes cell expansion and thus radicle protrusion (Yamaguchi and Kamiya, 2002). GA_3 appears not to be involved in the control of dormancy *per se* but promotes and maintains germination after the ABA-mediated inhibition of germination has been overcome (Bewley, 1997). The GA_3 treatment is effective in breaking dormancy by retarding metabolic processes that act to inhibit germination in seeds and initiates *de novo* production of GA_3 (Bewley and Black, 1994).

Seeds harvested from lower and middle siliques exhibited a higher germination percentage compared to those from upper positioned siliques. Pod position on the parent plant can affect the degree of dormancy of seeds (Bewley and Black, 1994). In seeds with morphophysiological dormancy, embryo growth and radicle emergence require a considerably longer period of time than in seeds with morphological dormancy (Baskin et al., 2000). The type of dormancy in *C. gynandra* is consistent with physiological dormancy since it was overcome by chilling, exogenous GA_3 application, and exposure to darkness.

In conclusion, the findings of this study demonstrate that fresh *C. gynandra* seeds are photosensitive with seeds exposed to more than 12 h of light having poor germination. This finding implies that covering the seeds with soil during planting rather than scattering on the surface could lead to improved seed germination in the field. Improved seed germination by pre-treating with 500 ppm GA_3 and chilling implies that commercial seeds could be treated with GA_3 or chilled before being sold to farmers. In this study, seeds collected from siliques positioned at the lower and middle portions of the reproductive part of the plant showed better germination than those harvested from siliques from the upper portions of the plant. Seeds from the upper portions of the plant tend to be harvested immature hence showing poor germination and therefore should be avoided when harvesting a seed crop.

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