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Full Length Research Paper

Light-induced transient dormancy in *Cleome gynandra* L. seeds

Julius O. Ochuodho[1*] and Albert T. Modi[2]

[1]Moi University, Chepkoilel Campus, P.O Box 1125, Eldoret, Kenya. [2]School of Agricultural sciences and Agribusiness (Crop Science) University of KwaZulu Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg.

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Events associated with dormancy release during seed germination still require explanation. The objective of this study was to examine seed responses during germination of *Cleome gynandra* in the presence or absence of light and at constant or alternating temperatures. Germination of *C. gynandra* seeds at 20°C was inhibited by light, but it was improved at 20°C in darkness. There was no photoinhibition when seeds were germinated at alternating temperature 20/30°C (16 h night and 8 h day). Photoinhibition was expressed more in seeds that were harvested late, after the pods had turned brown than in mature seeds at physiological maturity. It was more pronounced in seeds grown in extreme temperatures of 21/17 and 33/28°C, compared to those grown at 27/22°C. More than 5d photoinhibition reduced the germination of late harvested seeds such that the seeds did not completely recover their germination capacity. Photoinhibition is negative sensitivity to white light during seed germination in *C. gynandra*, likely controlled by the phytochrome system.

Keywords: Seed germination, photoinhibition, phytochromes, *Cleome gynandra*.

INTRODUCTION

Physiologically mature seeds are either dormant or non-dormant and will germinate once the dormancy is relieved or optimum conditions are provided. Environmental conditions may impose some dormancy condition on seeds and prolonged inhibition of germination may gradually enter a state of secondary dormancy (Karssen, 1995). In light requiring species, *Sisymbrium officinale* and *Arabidopsis*, light (Pfr) plays an important role in the biosynthesis of GAs and also increases the sensitivity of seeds to GAs (Hilhorst et al., 1986; Hilhorst and Karssen, 1988; Baskin and Baskin, 1998; Yamaguchi and Kamiya, 2002). These species germinated in darkness when exogenous GA was applied. Hilhorst (1990) suggested that the induction and breaking of secondary dormancy was phytochrome controlled in light-requiring seeds. This was also shown in guava seeds (*Psidium guajava*), where germination was induced by high R/FR ratio and alternating temperatures (Sugahara and Takaki, 2004).

Seed germination in many plant species is inhibited by continuous white light and such seeds germinate well in darkness (Bewley and Black, 1994) . Seeds of *Nemophila insignis, Amaranthus caudatus* and many cultivars of lettuce germinate normally in the dark but their germination is inhibited by light. Such seeds are referred to as negatively photoblastic (Gutterman et al., 1992; Bewley and Black, 1994; Baskin and Baskin, 1998). Photoinhibition involves the operation of phytochromes A and B, and its effectiveness depends on the duration of exposure and effluence rate (Bewley and Black, 1994; Franklin and Whitelam, 2004). It is also influenced by the seed coat. Takaki and Gama (1998) demonstrated the effect of seed coat on photoinhibition through chemical scarification of seeds of *Lactuca sativa* cv. Grand Rapids. Bewley and Black (1994) reported that seeds are susceptible to photoinhibition during cell elongation and this was supported by the observation that shoot elongation was inhibited by exposure to red light (Kato-Noguchi, 2002).

Dormancy in annual ryegrass was released through wet-stratification in darkness but a larger proportion of the seeds failed to germinate when wet-stratification was performed in the presence light at 5, 10, 15, 20, 25, 30 and 37°C (Steadman, 2004). *Cleome gynandra* exhibited negative photosensitivity on exposure to continuous white

*Corresponding author. E-mail: seedtechjo@yahoo.com

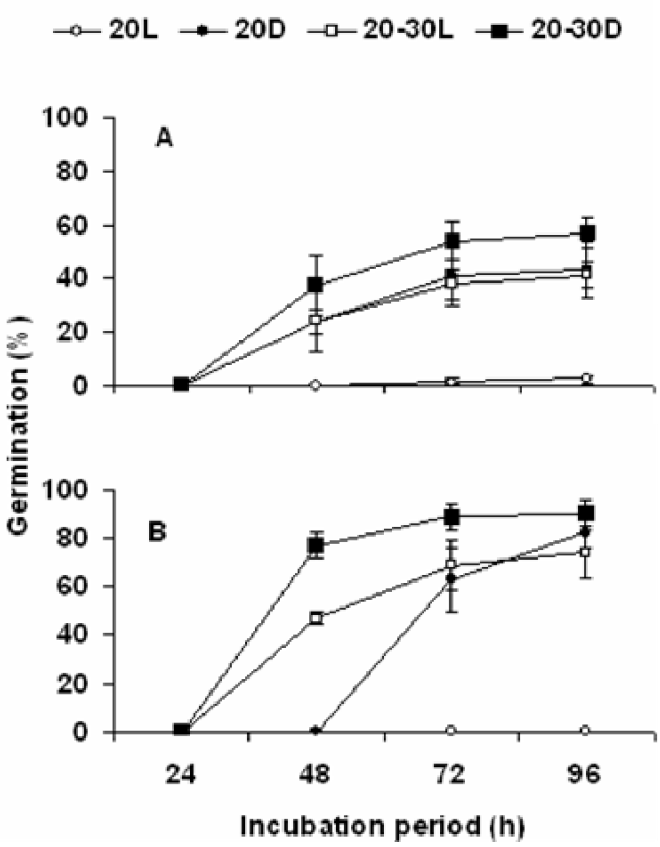

Figure 1. Germination of the original *Cleome* seeds from ARC (A) and KSC (B) at 20^{o}C or alternating 20/30^{o}C (16/8 h) in continuous white light or darkness. 20^{o}C with light ( ), 20^{o}C in darkness ( ), 20/30^{o}C with light ( ) and 20/30 ^{o}C in darkness ( ). Error bars represent SD, n = 6.

light at 20^{o}C during germination, but photoinhibition was greatly reduced at alternating 20/30^{o}C or at constant 30^{o}C (Ochuodho and Modi, 2005). The objective of the present study was to investigate the mechanism of photoinhibition during seed germination. The response of seeds harvested at different pod maturity stages to light and temperature interactions during germination was examined.

MATERIALS AND METHODS

Plant material

Two seed lots of *C. gynandra* were each obtained from Kenya Seed Company (KSC), Kenya and Agricultural Research Council (ARC), South Africa in 2002. Fresh seeds were produced from the KSC seed lot at the University of KwaZulu Natal farm in Pietermaritzburg. Pods were harvested separately at different maturity levels depicted by pod colour – mature green, yellow and brown. Other seed lots were grown in glasshouses at day/night temperatures of 21/16, 27/22 and 33/28^{o}C, and harvested when the pods had turned brown. The glasshouses experienced natural day length during the months of July to October 2002 (Pietermaritzburg: 29^{o}35 S30^{o}25 E) and controlled relative humidity of 60%.

Seed germination

Seeds from ARC and KSC were surface sterilised with 5% NaOCl for 10 min and then germinated at 20^{o}C or alternating 20/30^{o}C (16 h night/8 h day, respectively) in continuous white light or in darkness (Labcon LTGC 20-40; Johannesburg, South Africa). These seed lots were germinated also at 20^{o}C at varying light exposure periods of 0, 8, 12, 16 and 24 h. The seeds harvested from the field at diff-erent pod maturity stages were germinated at alternating 20/30^{o}C in darkness or continuous light. The seed lots from the glasshouses were germinated at alternating 20/30^{o}C in darkness for 10 days. Subsamples from these seed lots were incubated at 20^{o} C in conti-nuous light for 3, 5, 7 or ten days and then transferred to 20/30^{o}C in darkness for 10 days. In all the experiments, four replications of 50 seeds each were used. Seed was considered germinated when radicle protrusion was evident and germination percentage was determined 10 days after incubation.

Statistical analysis

Analysis of variance was used to compare the differences between seed lots ARC, KSC and those obtained from green, yellow and brown pods. GenStat statistical package (2000) was used to analyse germination percentages obtained and to compute standard deviations used to create the error bars.

RESULTS

Germination of seeds of different physiological maturity

Seeds from the ARC showed a germination of 57% and 41% at alternating 20/30^{o}C in darkness and in continuous light, respectively, after four days of incubation. Germination at 20^{o}C was 43% in the dark but it decreased to 2% in continuous light (Figure 1A). The seeds from KSC showed a similar germination pattern as the ARC seeds under the same conditions (Figure 1B). At alternating 20/30^{o}C, the KSC seeds had a germination of 90% in darkness and 74% in light, after four days. Seed germination at 20^{o}C was 82% in the dark, but the seeds failed to germinate in continuous white light. In both seed lots (ARC and KSC), the interaction between temperature and light was highly significant ($P < 0.001$).

The negative effect of light was also shown in mature fresh seeds harvested from green, yellow and brown pods grown in the field (Figure 2). Mature (black) seeds from green pods attained more than 90% germination within 96 h of incubation at alternating 20/30^{o}C in the presence of light or in darkness. The germination of seeds from yellow pods was intermediate, reaching about 70% in both light and darkness, after four days. However, the seed from brown pods showed the lowest germination of 65% in darkness and the germination was reduced to 35% in the presence of light. Analysis of variance showed there was a significant three-way interaction between exposure x lots x days ($P < 0.001$).

Germination response to light exposure period

There was significant difference in seed germination in

Table 1. Seeds of *C. gynandra* were produced in glasshouses set at different temperatures 21/16, 27/22 or 33/28^{o}C day/night, respectively. The seeds were harvested at brown pod maturity and germinated at alternating 30/20^{o}C day/night or 20^{o} C in darkness or continuous light. Another set of seeds was incubated at 20^{o}C in continuous white light for 7 d then transferred to alternating 30/20^{o}C in darkness for germination. Values represent means ± SD, n = 4.

Incubation condition	Temperature of production (day/night ^{o}C)		
	21/16	**27/22**	**33/28**
20/30 ^{o}C dark	76.0 ± 9.033	93.0 ± 3.464	83.5 ± 7.550
20/30 ^{o}C light	40.0 ± 5.888	53.5 ± 3.416	35.5 ± 1.915
20 ^{o}C dark	53.5 ± 8.226	76.0 ± 1.633	69.0 ± 7.605
20 ^{o}C light	0.00	0.00	0.00
7 days 20 ^{o}C light transfer to 20/30 ^{o}C dark	23.0 ± 6.831	39.0 ± 5.773	40.5 ± 5.291

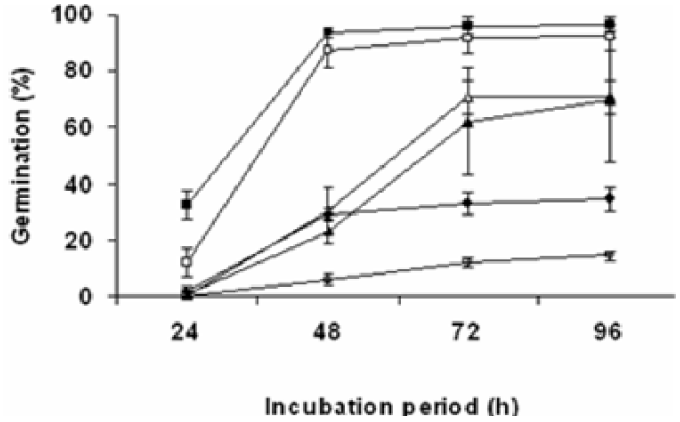

Figure 2. Germination of black seeds of *Cleome* grown in the field and harvested at different stages of pod maturity. The seeds were germinated at alternating 20/30^{o}C (16 h night/8 h day) in continuous white light or darkness. Seeds from green pods incubated in continuous white light ( ) or darkness ( ), yellow pods incubated in continuous white light ( ) or in darkness ( ) and brown pods incubated in continuous white light ( ) or in darkness ( ). Error bars represent SD, n = 4.

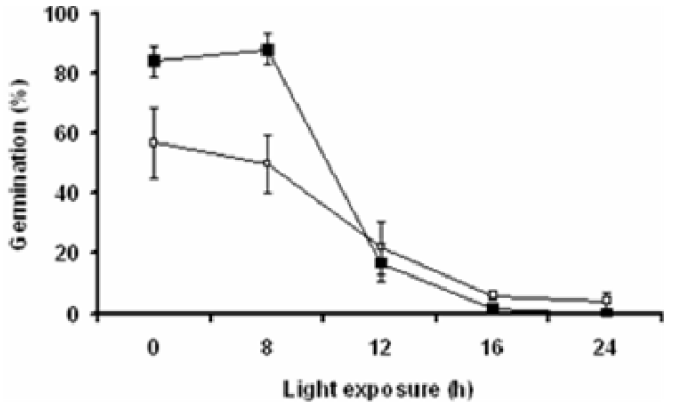

Figure 3. Seeds of *C. gynandra* were treated with white light at varying exposure periods during their germination at 20^{o}C. KSC seed lot ( ); ARC seed lot ( ). Error bars represent SD, n = 4.

response to varying light exposure periods (Figure 3). Germination reduced significantly (P< 0.001) when seeds were exposed to more than 12 h in a day com-pared to less exposure periods. The seeds either failed to germinate or only a few germinated when exposed to continuous white light at 20 ^{o}C.

Germination response of seeds produced at different temperatures

Seeds produced in the glasshouses at day/night temperatures of 21/16, 27/22 and 33/28 ^{o}C and harvested when the pods were brown had lower germination in the presence of light than in darkness (**Table** 1). The seeds produced at 21/16^{o}C showed 76% germination; those produced at 27/22^{o}C had a germination of 93% while those produced at 33/28^{o}C obtained a germination of 84% at the germination temperature of 20/30^{o}C after 4 days of incubation in darkness. The seeds showed better germination at 20^{o}C in darkness compared to alternating 20/30^{o}C in continuous light. The germination of seeds from 21/16, 27/22 and 33/28^{o}C was 54, 76 and 69%, respectively, after four days. However, the seeds failed to germinate at 20^{o}C in continuous light but on transfer to alternating 20/30^{o}C in darkness, the seeds had a germination of 23, 39 and 40% respectively.

Photoinhibition for 3 - 10 d significantly decreased seed germination compared to control (Figure 4) but the difference among photoinhibition treatments was not significant.

DISCUSSION

The results show that the germination of *C. gynandra* seeds was negatively photosensitive and the greatest effect of photoinhibition was observed at 20^{o}C (Figure 1 and Table 1). It had been shown that photoinhibition of germination in *Cleome* was pronounced at 20^{o}C, but minimally expressed at optimum germination temperatures (Ochuodho and Modi, 2005) . Gutterman et al. (1992) and Steadman (2004) obtained similar results in *A. caudatus*

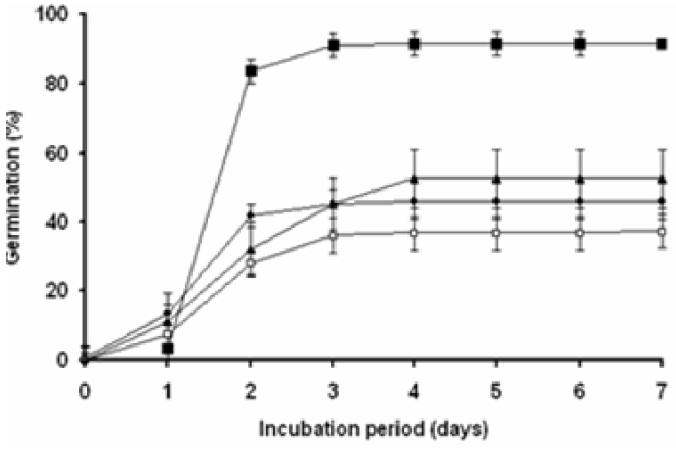

Figure 4. Seeds of *C. gynandra* were produced in the glasshouse set at day/night temperatures of 27/22^{o}C and harvested when the pods were mature brown. The seeds were germinated at alternating 20/30^{o} C in darkness for 7 days after incubation at 20^{o}C in continuous white light (photoinhibited) for 3 d ( ), 7 d ( ), 10 d ( ) or planted direct ( ). Error bars represent SD, n = 4.

and annual ryegrass, respectively. Gutterman et al. (1992) showed that the inhibitory effect of light on germination of *A. caudatus* seeds was enhanced in low oxygen or at low temperatures.

Germination can be delayed, reduced or may fail to occur depending on the physiological status of the seeds and, the light and temperature at which the test is carried out (Figure 1 and Table 1). The results showed that delayed harvesting and temperature during seed production influenced photoinhibition (Figure 2 and Table 1). There are many reports that physiological maturity is reached after maximum seed dry mass in several plant species (Hilhorst and Toorop, 1997; Still and Bradford, 1998). Ochuodho (2005) showed that physiological maturity in *C. gynandra* was attained just before yellow pod stage. The black seeds harvested from green mature pods had attained physiological maturity and were not adversely affected by photoinhibition. Seeds harvested at the brown pod stage showed strong negative response to continuous light during germination. The temperature at which seeds were produced influenced the quality of seed, especially, their germination (Table 1). The seeds with the lowest germination were produced at the lowest temperature of 21/16^{o}C and were also the most photosensitive. These results support observations made by Steadman et al. (2004) in annual ryegrass that seed maturation environment, particularly temperature, had significant effect on seed numbers and seed dormancy characteristics.

Photoinhibition is referred to as pseudo-dormancy, where seeds fail to germinate because conditions of incubation are not optimum. The seeds are in a state of suspended germination and the effects of light are modulated by temperature and light exposure period (Figures 1 – 3) . The seeds of *C. gynandra* that were incubated at 20^{o}C in continuous white light either failed to germinate or showed very low germination (Figure 3). A large propor-tion of annual ryegrass seeds failed to germinate after wet-stratification in light, even at low temperatures (Ste-adman, 2004). According to Bewley and Black (1994) photoinhibition of seed germination by prolonged illumi-nation is a manifestation of high irradiance reaction of photomorphogenesis. Similar conclusions were made by Thanos et al. (1994) working with *Matthiola tricuspidata* and, Franklin and Whitelam, (2004) using *Arabidopsis*. It is postulated that this negative photosensitivity at 20^{o}C in continuous white light is due to phytochrome imbalance, especially ABA/GA content. The biosynthesis of gibberellins, which is mediated through the phytochrome sys-tem, may have been inhibited by light exposure (Baskin and Baskin, 1998; Kato-Noguchi, 2002). Photoinhibited seeds recovered their germination capacity after treat-ment with GA_3 (Ochuodho, 2005), supporting the involve-ment of GA in this phenomenon. The seeds that were photoinhibited did not fully recover their germination cap-acity after being transferred to optimum germination con-ditions (Table 1 and Figure 4). This is in contrast to the findings by Hills et al. (2001) with thermoinhibited seeds of *Tagetes minuta* and cold acclimated plants of *Arabi-dopsis thaliana* (Gray et al., 2003), which germinated fully when transferred to optimum conditions. Therefore, seed dormancy differs from photoinhibition because dormancy has to be relieved through external treatment before seed germination can occur. Photoinhibition has been defined as 'conditional dormancy' (Baskin and Baskin, 1998) and because conditional dormancy is progressive, some of the seeds could have attained secondary dormancy and thus failed to germinate. This has been explained as an adaptation by many small seeded weedy species to sur-vive adverse conditions.

The seed coat has been reported to play a partial role in photoinhibition (Gutterman et al., 1992), in the induct-ion of secondary dormancy in *A. caudatus* (K pczy ski and Bihun, 2002) and to influence seed dormancy, germination and longevity in *Arabidopsis* (Debeaujon et al., 2000). Scarification of the seed coat was shown to improve the germination of phytochrome-controlled seed germination (Gutterman et al., 1992; Takaki and Gama, 1998; Ochuodho, 2005). Scanning Electron Microscopy analysis showed that the seed coat of *C. gynandra* is similar to that of *Arabidopsis* (Ochuodho, 2005).

Poor quality seeds are usually given some pretreatments such as chilling and priming to improve germination (Hardegree, 1996; McDonald, 1999). Chilling is carried out at temperatures below the minimum required for germination (usually 5 ^{o}C), while priming is done at optimum temperatures. Seeds of *C. gynandra* that were chilled at 5 - 10^{o}C in light for 5 days showed the same germination as non-chilled (Ochuodho and Modi, 2005). It can therefore be stated that while pre-chilling generally improves seed germination, the light condition in which

pre-chilling is performed could be important, especially when dealing with negatively photosensitive species as observed in annual ryegrass and *C. gynandra*. Hilhorst and Karssen (1988) demonstrated the dual effects of light on nitrate stimulated seed germination in *S. officinale* and Copeland and McDonald (1995) stated that seeds that are sensitive to light are also sensitive to KNO_3 while ISTA (2004) recommend their pre- treatment. However, *C. gynandra* showed no sensitivity to KNO_3 (Ochuodho, 2005), which further confirms its negative photosensitivity.

In conclusion, these results indicate that the seeds of *C. gynandra* are negatively photoblastic. The seeds responded negatively to continuous white light during germination at 20^{o}C, when light exposure beyond 12 h drastically reduced germination. That photoinhibition was more evident in seeds harvested after physiological maturity (when the pods had turned brown) and was much less or absent in seeds at physiological maturity. The condition in which seeds are produced also influenced the extent of photoinhibition. Photoinhibited seeds did not completely recover their germination capacity when transferred to optimum germination conditions, except after treatment with GA_3. Photoinhibition of seed germination can be explained as a reaction controlled by the phytochrome system.

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