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Association of seed coat colour with germination of three wild mustard species with agronomic potential

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Seed dormancy and germination present significant challenges when wild species are domesticated for cultivation and economic exploitation. Wild plant species are generally characterized by dormant seeds with variable germination widely spread over time. The objective of this study was to evaluate the influence of colour selection on seed germination of wild mustard (Brassicaceae*)* species that have been identified as wild edible leafy vegetable in South Africa. Seed lots were separated by colour and germinated in a completely randomized design (CRD) after chilling and after-ripening for 6 months. The light seed lot of cultivar *Kwayimba* (K) showed higher germination percentage than the dark seed lot of the same cultivar but colour selection did not improve the germination in cultivars *Isaha* (I) and *Maslahlisane* (M). The dark seed lot of K recorded the lowest germination percentage and the slowest germination rate. Chilling improved the speed of germination in wild mustards, but after-ripening had no effect. Seed colour change in wild mustards intensifies after physiological maturity and may be accompanied with weight increase or not. The seed coat colour may not be a good indication of the physiological status of the seed but together with physiological tests (germination) can give insight on the quality of a seed lot.

Key words: Seed colour, image analysis, seed germination.

INTRODUCTION

Diversification through domestication of locally adapted wild species of known nutrient quality has been suggested as more appropriate and could improve food security (Jansen van Rensburg et al., 2004). However, wild plant species are generally characterized by dormancy and variable germination behaviours that hinder or slow down their cultivation. The seeds show wide morphological variations including colour, and colour selection has been recommended as a quick method of improving seed quality**.**

Seed colour has been reported to play a role in seed dormancy and germination, as seeds attain their specific colour at physiological maturity (Powell, 1989; Ochuodho, 2005). Seed coat pigmentation and structure have been shown to influence germination (Debeaujon and Koornneef, 2000; Debeaujon et al., 2000). In *Arabidopsis*, structural mutants lacking some seed coat layers and those that showed less pigment impregnation were lighter and germinated better than wild types (Debeaujon et al., 2000). While dark coloured testa accompanied with slow water uptake was attributed to the presence of phenolic compounds and tight adherence of the seed coat to the embryo in legumes, some dark soybean cultivars whose seed coats are loosely attached to the embryo showed greater rate of imbibition and fast germination (Chachalis and Smith, 2000). There were no differences detected in

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the content of phenolic material between permeable and impermeable seed coats of the accessions with dark coloured seeds (Chachalis and Smith, 2001).

However, the permeable seed coat was shown to have high density of deep and wide pores. Atanassova et al. (2004) showed that, seeds of 3 anthocyaninless mutants in tomatoes germinated faster than the wild type. The inner epidermal testa layer in these mutants did not have condensed tannins that contributed to the rigidity of cell structure, thereby reducing permeability. Light coloured seeds of radicchio were shown to have reduced germination and as seed colour became darker, seeds showed higher, faster and more uniform germination (Pimpini et al., 2002). The colour difference resulted from incomplete seed development as evidenced by lower seed weight and smaller embryos. Coste et al. (2005) found good correlation between pod colours measured in hue angles by spectrophotometer and seed water content. In this way, the authors were able to estimate physiological maturity and separate the pods and seeds. It is known that, seed germination of weedy species is highly variable and our observation was that, seeds of different colours and colour intensity showed varying germination percentages. The aim of this study was to evaluate the association of seed colour with the germination in 3 wild mustard cultivars.

MATERIALS AND METHODS

Plant materials

Seeds of wild mustard were produced under rainfed field conditions at Umbumbulu, 65 km South of Pietermaritzburg and mature dry seeds were harvested when the pods were brown. Three local vegetables known as *Isaha* (I), *Kwayimba* (K) and *Maslahlisane* (M), were used. M and I were originally collected from KwaZulu-Natal, while K was obtained from Eastern Cape Province. The seeds were visually selected on the basis of colour difference while shining white light on the seeds placed on a dark background and divided into light and dark components (seed lots). This selection produced 6 seed lots, which were each packaged in separate paper bags. The seeds were germinated directly or after prechilling at 5 to 10°C for 5 days or after storage in dry rooms with controlled temperatures of 5 and 20°C for 6 months.

Image analysis

The seed lots were analysed with AnalySIS$^{(R)}$, computer software for soft image analysis that determines the exact quality of colour by the wavelength (hue or colour), the saturation (colour purity) and the intensity (brightness). Using the software 50 seeds (positions) were randomly chosen and focused on for colour determination within a sample of 400 seeds. The program quantified the colour and the pixel values obtained were averaged.

Seed germination

Four replications of 100 wild mustard seeds were placed in Petri dishes (10 cm diameter) on 3 Whatman No.1 filter papers moistened with 7 ml of distilled water. The Petri dishes were arranged in a completely randomized design (CRD) and were incubated for 10 days in growth chambers (Labcon, LTGC 20-40; Johannesburg, South Africa) at alternating temperature 30/20°C day/night (8/16 h) (Ochuodho and Modi, 2005). Seeds were considered germinated when the radicle protruded and germination counts were performed daily in order to calculate mean germination time (MGT) according to De Villiers et al. (2002). Seedling evaluation was performed on the tenth day based on ISTA (2004) guidelines for the *Brassicas;* normal seedlings have primary root, hypocotyl and cotyledons with terminal buds intact. Figures were developed from germination data for 7 days because the results were not different with those for 10 day. Germination tests were carried out before and after visual selection of the seed lots.

RESULTS

Image analysis

It was difficult to distinguish the cultivars visually on colour basis and soft image analysis showed that, there were no significant differences between the 3 cultivars. However, on visual selection coupled with image analysis, the colours of the dark seed lots were not significantly different but cultivars K and I were closer (Figure 1). While the colours of the light seed lots of cultivars K and I were not different, they were significantly different ($p < 0.001$) from M. This colour difference was brought out clearly by both the hue and saturation but not colour intensity. The light and dark seed lots of cultivar K appeared different visually and were almost significantly different ($p < 0.05$) in hue. Table 1 showed that, 70% of the seeds of cultivars K and I were dark and heavier. On the contrary, the same number of seeds of the light and dark seed lots of cultivar M had the same weight but the proportion (in number) of the dark component was higher than of the light.

Seed germination

The germination percentage of cultivar I and M were not different ($p > 0.05$) but were significantly higher than the germination of K. Cultivar M showed the fastest germination having attained > 70% germination after 1 day. Colour selection did not change the germination percentage of the cultivars M and I but the rate and percent germination improved in the light seed lot of K (Figure 2). The light seed lot of K showed a high germination percentage similar to those of cultivars M and I.

Dark M showed the fastest germination having a MGT of 1.5 days while dark K showed slow germination speed of 3.5 days (Figure 3). On the other hand, the light seed lots of all the cultivars showed similar MGT of 2 days. Pre-chilling the seeds at 5 to 10°C for 5 days showed a significantly increase in the speed of germination (Figure 4) as all the seed lots showed > 40% germination after day one. However, after-ripening at 5 and 20°C did not

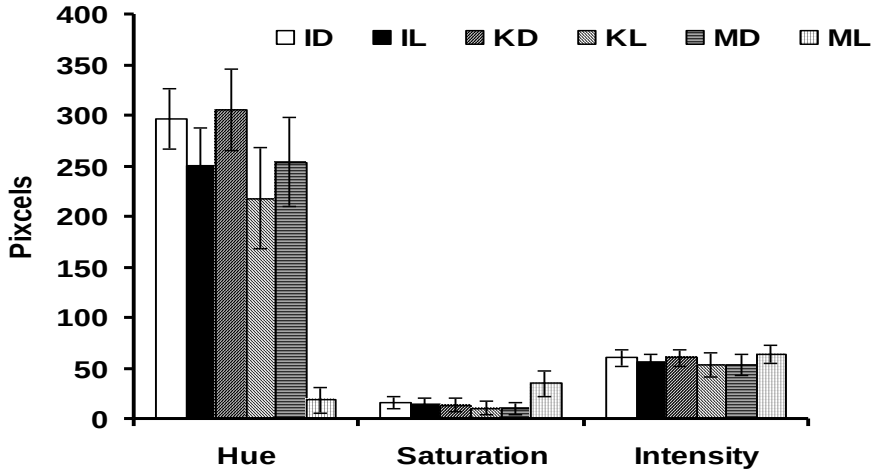

Figure 1. Colour analysis of seeds of wild mustard cultivars I, K and M by analysis after visual selection into light and dark coloured seed lots. ID, IL - dark and light I; KD, KL - dark and light K; MD, ML - dark and light M. The seed lot was spread out and between 50 points (seeds) analysed and the average obtained. The error bars represent SD, n = 50.

Table 1. Ten sub samples of 400 seeds were visually separated into light and dark colour components and seeds were counted [proportion (%)] and 100 seed weight obtained [Weight (g)].

Cultivar	Seed lots	Proportion (%)	Weight (g)
I	Light	28.3 ± 5.01	91.91 ± 5.78
	Dark	71.7 ± 5.32	103.19 ±0.60
K	Light	30.2 ± 4.96	85.85 ± 6.43
	Dark	69.8 ± 5.03	106.11±0.54
M	Light	44 ± 3.79	99.64 ± 2.92
	Dark	56 ± 3.44	100.29±0.87

The error values represent SD, n = 10.

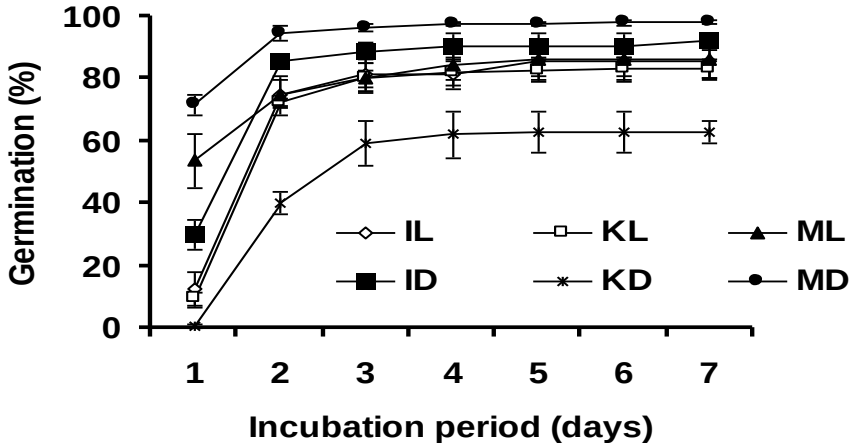

Figure 2. Germination percentage of the wild mustard cultivars I, K and M after visual selection into light (L) and dark (D) coloured seed lots. Seeds were incubated at alternating temperatures 20/30°C and 16/8 h, night/day, respectively. Seedling evaluation was done after 10 days. Error bars are standard deviation, n = 4.

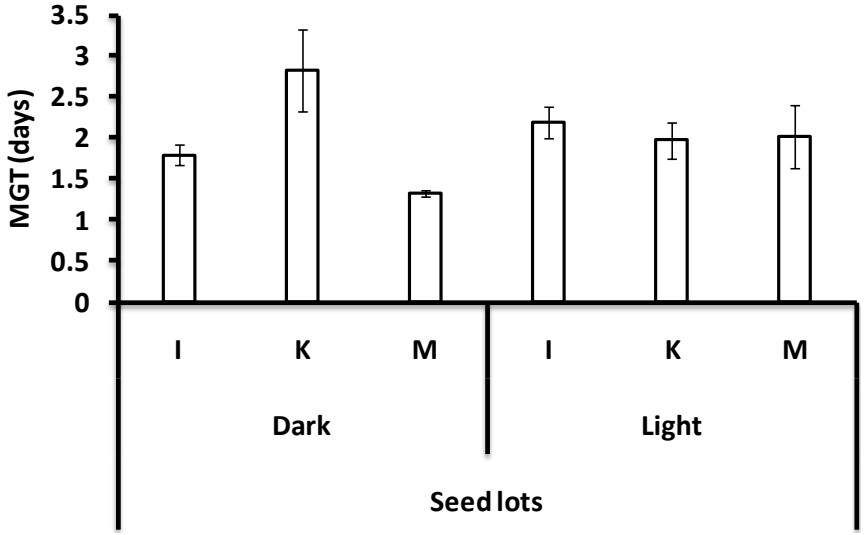

Figure 3. The speed of germination was computed as mean germination time (MGT) of the cultivars of wild mustard I, K and M before and after visual selection. The seeds were germinated at alternating temperatures 20/30°C and 16/8 h, night/day, respectively. Seeds that have germinated were counted every day for 10 days and MGT was calculated. Error bars represent SD, n = 4.

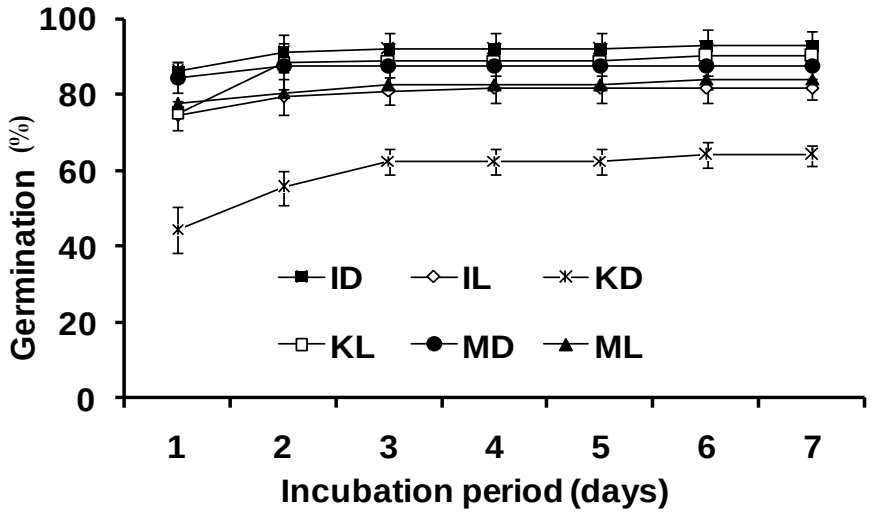

Figure 4. Germination of the seeds of cultivars of wild mustard I, K and M after visual selection into dark (D) and light (L) coloured seed lots. The seeds were germinated at alternating temperatures 20/30°C and 16/8 h, night/day, respectively after 5 days pre-chilling at 5 to 10°C in darkness. Seeds that have germinated were counted every day for 10 days.

improve the germination of cultivars M and I (Table 2) but by the fourth month there was marked decrease in the germination of cultivar K.

RESULTS AND DISCUSSION

It was not possible to separate the cultivars by colour with soft image analysis initially but after visual selection the hue and saturation were significantly different between the seed lots of cultivar M. While Coste et al. (2005) suggested that, hue angle measured on pods be used to monitor seed drying, hue (pixels) was used in this study to distinguish the seed lot colours. Hue is the measure of true colour (by wavelength) and this can be influenced by phenolics in *Arabidopsis* (Chachalis and Smith, 2000),

Table 2. Seed lots of wild mustard were stored in dry temperature controlled rooms at 5 and 20°C for six months and germinated at alternating temperatures of 20/30°C, 16/8 h night and day respectively. Germination percentage was scored daily for 10 days. Error values represent SD, n = 4.

Storage Temperature (°C)	Storage Period (month)	Seed lots					
		ID	IL	KD	KL	MD	ML
5	Control	93 ± 2.65	83 ± 5.57	57 ± 7.02	79 ± 2.65	95 ± 3.00	85 ± 2.52
	1	92 ± 3.42	81 ± 7.55	57 ± 6.19	79 ± 5.89	95 ± 3.30	83 ± 6.63
	2	96 ± 4.76	78 ± 7.83	40 ± 11.00	73 ± 5.74	97 ± 4.76	84 ± 8.16
	3	95 ± 2.58	88 ± 3.42	33 ± 5.03	66 ± 2.83	97 ± 2.52	88 ± 1.00
	4	90 ± 1.63	85 ± 8.70	32 ± 5.97	61 ± 5.74	92 ± 3.65	89 ± 3.42
	6	93 ± 1.91	82 ± 3.46	33 ± 7.72	63 ± 5.74	94 ± 1.03	89 ± 1.15
20	1	94 ± 3.65	78 ± 9.10	42 ± 14.84	71 ± 6.23	91 ± 3.00	87 ± 3.46
	2	93 ± 2.58	82 ± 1.91	45 ± 9.56	76 ± 2.58	96 ± 2.58	91 ± 4.76
	3	95 ± 2.52	85 ± 5.03	43 ± 4.16	73 ± 4.12	89 ± 3.00	90 ± 4.32
	4	95 ± 3.00	83 ± 3.83	37 ± 11.50	65 ± 9.59	94 ± 1.63	89 ± 4.76
	6	90 ± 3.27	79 ± 5.26	19 ± 3.46	58 ± 4.12	94 ± 2.83	87 ± 6.19

anthocyanins in soybean (Atanassova et al., 2004) or seed maturity stage in radicchio (Pimpini et al., 2002) and in *Cleome gynandra* (Ochuodho, 2005). On weight basis, the light and dark components of cultivar M were the same, while the dark component of cultivar K and I were heavier. These observations seem to suggest that, seed colour may not be a dependable determinant of the physiological state or maturity stage of these species.

The high germination percentage shown by the light and dark seed lots of M indicated that, the dark colouration did not negatively affect seed germination. These observations could imply that, the dark colour in cultivar M set in later after physiological maturity and the colour change did not affect seed mass. This observation is contrary to that by Debeaujon et al. (2000) and Chachalis and Smith (2001) that the dark coloured seeds showed poor germination. This was explained by the structural and morphological differences observed in the seeds studied then, but the present study did not go that far. The seed coat colouration in cultivar M did not influence germination as was observed in some accessions of soybean by Chachalis and Smith (2001). The light coloured seed lots of cultivars K and I weighed less compared to the dark seed lots, and yet they showed higher percent germinations. Pimpini et al. (2002) observed that, light coloured seeds of radicchio were immature and Ochuodho (2005) made the same conclusion with *C. gynandra*. It is possible that, the dark colouration intensified after physiological maturity or the climatic condition caused the darkening of wild mustard seeds.

However, in cultivar K, as the dark colour developed, seed mass increased and germination percentage decreased. This confirms further the observation made earlier that, the dark coloration intensified after physiological maturity, either through chemical action or impregnation of compounds. These observations are in line with those by Hilhorst and Toorop (1997) that in some crucifers, maximum seed mass is attained after physiological maturity. The seeds that failed to germinate were fresh and tetrazolium test showed that, they were live. It is probable that secondary dormancy may have been induced in this cultivar. This finding is supported by Ochuodho (2005) who concluded that, prolonged stay of *C. gynandra* seeds in the field after physiological maturity induced secondary dormancy, a common phenomenon in weedy species. Furthermore the seed lots showed similar proteins at varying band intensity (data not shown), another factor supporting the earlier observation that all the seed lots had attained physiological maturity.

High imbibition and germination rates in dark soybean seeds were attributed to lose seed coat attachment to the embryo (Chachalis and Smith, 2000) and higher numbers of large pores (Chachalis and Smith, 2001). The morphology of seed (seed coat) was not examined in this study but the light seed lot of cultivar K (KL) showed faster imbibition and absorbed more water (Ochuodho and Modi, 2008). Pre-chilling improved percent germination slightly but significantly increased the rate of germination MGT in all the seed lots. This could be because pre-chilling allowed all the seed lots to imbibe enough water to facilitate germination. The high rate of germination could also be due to an increase of bioactive GA in the embryo during pre-chilling (Poljakoff-Mayber et al., 2002; Perez-Flores et al., 2003) and as GA increased, ABA decreased because of enhanced catabolism (Jacobsen et al., 2002; Gonai et al., 2004). When the seeds of cultivar K were treated with exogenous GA_1, they showed significant increase in germination speed and total germination percentage (Ochuodho and Modi, 2008). However, 6 months of after-ripening did not improve germination percentage but

decreased the germination of cultivar K. This seems to support the argument that the seeds were harvested after physiological maturity and hence did not require after-ripening. The behaviour of cultivar K was found to be contrary to the statement by Copeland and McDonald (1995) that secondary dormancy was broken by low-temperature stratification and storage at 20°C, among other treatments.

Conclusion

In conclusion, colour selection resulted in increased seed germination in cultivar K but not in cultivars I and M. All the light coloured seed lots showed high germination percentage and one dark seed lot, which was heavier, showed poor germination. The seeds of the wild mustard cultivars turned black after physiological maturity but only cultivar K may have developed secondary dormancy as it persistently showed low germination. There was no clear relationship between seed colour and seed germination, and therefore colour selection may be an added cost to seed processing without adding value to seed quality.

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