

INHERITANCE OF ALBINISM IN CHICKPEA

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ABSTRACT

Albinism in chickpea (*Cicer arietinum* L.) was detected in an $M_{4,6}$ population after seed of the M_0 generation had been subjected to a 30 kR gamma ray treatment. Observations in succeeding generations showed that it was controlled by one recessive gene. The gene was designated the symbol *a-1*. The albino plants died before seed set could take place and the allele is therefore to be maintained in the heterozygous combination of *a-1 A-1*. The phenomenon of albinism was studied at the cellular level and several possible uses of the albino trait are discussed.

INTRODUCTION

Most chickpea breeders and geneticists have seen once in a while an albino chickpea plant and considered it a freak phenomenon. Scientists involved in mutation breeding have come across it more frequently (van Rheenen *et al.*, 1993). However the inheritance of albinism has not been published according to our literature search in SATCRIS data base 1996; AGRICOLA 1984 to 1996; and Muehlbauer and Singh 1987. This can be explained by the fact that the albino trait is lethal and the plants die at an early stage. A study of the inheritance of albinism could be possible though if plants, heterozygous for the albino character, were identified and the segregating progenies grown.

The authors observed in field experiments during 1994-95 several plots with a relatively large number of albino plants. They used this opportunity to study albinism in chickpea from a genetic and cellular point of view, realizing that the trait is of importance for the study of pathways of chlorophyll synthesis and for protoplast fusion in plant breeding efforts.

MATERIAL AND METHODS

Seeds of the Kabuli chickpea variety 'ICCV 2' were exposed in 1986 to a treatment with 30 kR gamma rays as described by van Rheenen *et al.* (1993). The M_1 was grown

during 1986 and the M_2 in 1987. Out of the M_2 population one plant was selected for its relatively large leaf size and designated ICCV 2B-30KR-LL. Its M_3 progeny was grown in 1988. The seeds were bulked and the $M_{2,4}$ generation sown in a wilt-sick plot to screen for multiple disease resistance in 1969. Single plant selections were made which were evaluated in 1990 in progeny rows for general performance. Again the progeny seed was bulked and used for sowing a replicated yield trial in 1994. We noted in the plots of 1994 an unusually large number of yellowish plants that died well before flowering. The yellowish and normal green plants in the plots were counted and the green plants of one plot were harvested separately. In 1995 we raised the progenies and recorded the number of green and albino plants in each progeny. Out of the 188 entries we selected three that showed segregation for the albino character and harvested these three separately for further use. In 1996 we germinated 100 seeds of one of these on wet filter paper in petridishes of 16 cm diameter under normal light conditions in a laboratory. After 5 days the seedlings were inspected and transferred to four 25.5 cm high and 30 cm wide pots filled with vertisol soil. Three days later we selected plants for electron microscopic studies. We excised 5 mm segments from the mid rib region of the leaves and immediately placed these in vials containing

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2% Glutaraldehyde in 7.2 pH 0.1 M phosphate buffer. The samples were deaired under vacuum till all the leaf segments settled to the bottom of the vials and left overnight. The samples were thoroughly washed with filtered distilled water and post-fixed in 4% aqueous Osmium tetra oxide for 4 hrs. The post-fixed samples were washed several times in filtered distilled water and dehydrated serially in 30-100% ethanol. The samples were left overnight in 100% ethanol and then embedded in spun to form blocks (Reddy *et al.*, 1991; Reddy *et al.*, 1995). The blocks were cut into sections of about 70 nm thickness with LKB Reichert Jung Ultramicrotome using glass knives having boats to hold water. The floating sections were transferred onto 40 mesh copper grids pre-coated with 70 nm carbon film using JSM-

2000 vacuum coating unit and stained with Lead Citrate and Uranyl Acetate (Hayat, 1972) and observed under Philips CM20 TEM. Photographic exposures were made with a plate camera.

As the albino symptoms resembled those of iron chlorosis, we sprayed the plants of 2 pots with a 0.25% solution of Ferrosulphate, and we provided those of one other pot with a glucose solution of 10 g in 1.5 L.

The count data were statistically analysed by the χ^2 method.

RESULTS AND DISCUSSION

The albino plants in the field looked golden yellow (Fig. 1). However, in petridishes for five days and, transferred to pots for another



Fig. 1. Albino (left) and green chickpea plant (right)

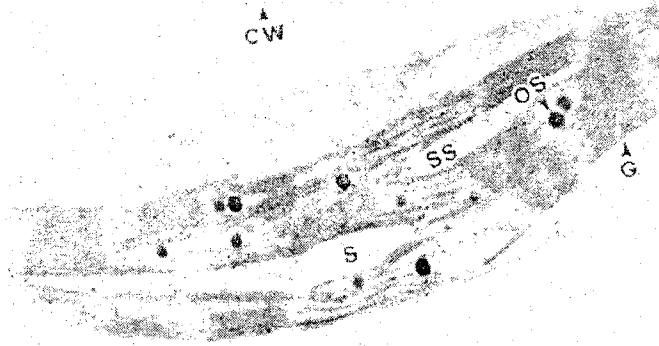


Fig. 2 (a) Green tissue section showing cell wall (CW), grana (G), stromatal spaces (SS) and osmophytic bodies

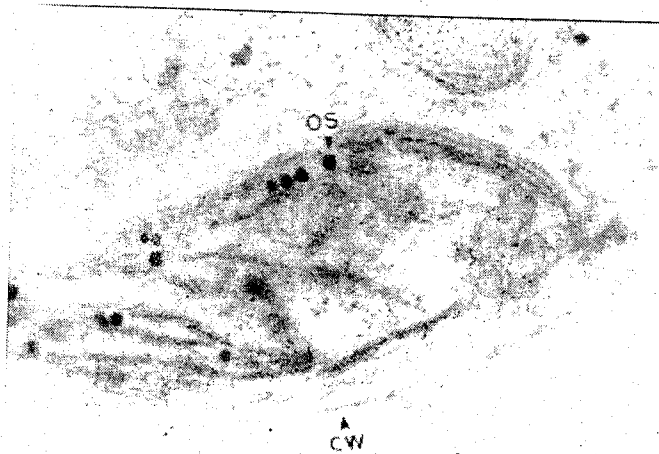


Fig. 2 (b) Section of albino leaf tissue showing cell wall (CW), osmophytic bodies (OB) and large stromatal space

Fig. 2. Electron micrographs of chloroplast of green (x 30K) and albino (x 22.5K) leaf tissues

two days, no difference was noted between albino and normal seedlings as all looked green. However on day 8, plants started to show the albino character. They continued their growth, but on day 19 they had died. The FeSO_4 and glucose treatment had no visible effect on albinism. The chloroplasts of the green and albino plants looked different as shown in Fig.

Table 1. Segregation data of albinism in chickpea

Year	Generation	Segregation for plant color			Expected ratio ²	χ^2	P
		Green	Albino	Segregating			
1994	M _{4,5} : plants	393	72	-	5:1	0.468	0.50
1995	M _{6,7} : progenies	53		27	3:2	1.303	0.26
	M _{6,7} : plants ¹⁾	551	168	-	3:1	1.124	0.29
1996	M _{6,8} : plants	78	19	-	5:1	0.563	0.47

¹⁾ Segregating progenies

²⁾ If M₄ is heterozygous *A/-I a/-I*, M_{4,5} will segregate: 1 *A/-I A/-I* : 2 *A/-I a/-I* : 1 *a/-I a/-I*; *a/-I a/-I* will die, not producing seed; M_{4,6} then will produce: [4 *A/-I A/-I*] + [2 *A/-I A/-I* + 4 *A/-I a/-I* + 2 *a/-I a/-I*], giving a ratio of 5 green : 1 albino; similarly, M_{6,8} will show a 5 : 1 ratio. The M_{6,7} progenies will have derived from (4+2) *A/-I A/-I* : 4 *A/-I a/-I* plants, yielding a 3 : 2 ratio of non-segregating : segregating progenies; the segregating progenies will show a ratio of 3 : 1 green : albino.

2 a and b.

The plant counts in different generations conducted during 1994, 1995 and 1996 are summarized in Table 1, and suggested that the albino trait to be recessive and controlled by one gene. In accordance with common genetic nomenclature we propose the gene symbol *a/-I* for albino and *A/-I* for green, the same symbol that has been used in *Neurospora*, where three genes have been identified for albinism (Carattoli *et al.*, 1991).

The observation that the plants germinated in petridishes and transferred to pots stayed green for a week suggests that the requirement for chloroplast formation can be met from the cotyledons for that period of time but no longer.

The failure of FeSO₄ to restore the green color in the albino plants suggests that in this case albinism is not related to iron chlorosis.

The microscopic studies suggest that the structure of the chloroplasts in the case of green plants is well organized (Fig. 2a) with large intact grana, starch granules, osmophylic bodies, and well formed stomatal spaces

indicative of photosynthetic activity (Thakur *et al.*, 1995). In comparison, the chloroplasts of the albino plant leaves are devoid of well organized grana, and have few vacuoles, membrane filaments and osmophilic bodies (Fig. 2b).

There are several areas where albinism has been useful as research tool. For example, in the study of fruit ripening in *Capsicum* (Newman *et al.*, 1989), and in the analysis of the pathway of carotenoid synthesis in *Arabidopsis* where two genes designated *pds 1* and *pds 2* disrupted phytoene desaturation (Norris *et al.*, 1995). Albinism was also used in chloroplast genome mapping and plastid structure analysis in soybean (Lee *et al.*, 1989). Not only for basic but also for strategic research albinism has been utilized. In lettuce breeding for instance a parent, homozygous for a recessive albinism gene and heterozygous for a dominant kanamycin resistance gene yielded "universal hybridizer protoplasts" for fusion with wild species' protoplasts (Chupeau *et al.*, 1994). Similarly albino tomato and normal green potato plants were used for protoplast fusion by Wolters *et al.* (1995). For chickpea the same technique could be useful, as several

wild *Cicer* species harbor important resistant traits and two are cross-incompatible with the cultivated species. character was simply inherited and is easy to maintain.

The literature suggests that albinism can be controlled by different genetic systems (Branch and Kvien 1992; Dwivedi *et al.*, 1984; Long *et al.*, 1993; Zubko and Day 1998). In the present study of chickpea the albino

On request seed can be made available from Genetic Resources and Enhancement Program, ICRISAT that will produce a population segregating for green and albino plants in an expected ratio of 5:1.

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