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Regeneration and Somaclonal Variation in *Medicago sativa* and *Medicago media*

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Abstract: Four cultivars of *Medicago sativa* L. (Dupuits, Spreado-2, Roamer and Rangelander) and one cultivar of *M. media* (Rambler) were tested for regeneration capability. 100 plants of each cultivar were tested. Calli were induced from explants either of petiole from adult plants or seedings grown in sterilized conditions. Cultivar Rambler was found highly embryogenic and gave the highest regeneration percentage (26%) followed by 17, 14, 10 and 7%, respectively for Rangelander, Spreador-2, Roamer and Dupuits. Somaclonal variations were observed at the cellular level for chlorophyll and anthocyanin synthesis and at the whole plant level for ploidy level i.e. vigorous growth larger leaf size.

Key words: Regeneration, somaclonal variation, Medicago species, adult plant, seedings

Introduction

The regeneration of plants from callus and suspension cultures is essential for the straightforward application of somalonal variation, complementing existing breeding programs to crop improvement. The regeneration capability of plants varies from species to species, cultivar to cultivar and plants to plants. The regeneration is also affected by several other factors: Inoculum, the age and health of donor plant, Environmental factors e.g. temperature and light and Medium composition, particularly the combination of growth regulators (Pierik, 1987). In general regeneration can be accomplished by micro propagation, in which tissue organization is maintained i.e. clonal multiplication of meristems culture and via embryogenesis or organogenesis from disorganized cells (suspension, callus and protoplast cultures). The present study was undertaken to determine the frequency of regenerating calli and accomplishment of whole plants between species, cultivars and within the cultivar between different anatomical sources of explants of adult plants and sterile germination seedlings of cultivars of *Medicago* species.

Materials and Methods

Establishment of callus culture: Two different sources of explants were used to produce calli. The perioles were excised from the Green house grown plants. Seeds and petioles of *Medicago sativa* L. cvs. Dupuits, Spreador-2, Rangelander and Roamer and Medicago media cv. Rambler were surface sterilized by shacking with 5% Domestos bleach solution for 10 min. The seeds and petioles were washed four times with sterile water. The seed were than transferred to pre-sterilized petri dishes containing moist Whatman no. 3 filter paper and incubated in the dark at 25°C for germination. While petioles' explants were transferred to Murashige and Skoog (1962) medium (MS medium) containing 2 g m^{-3}2,4-dichlorophenoxyacetic acid and 0.25 g m^{-3} kinetic, supported by 0.9% w/v agar, then incubated at 25°C in the dark. After 3 days explants (radicle, hypocotyl and cotyledon) from seeding grown in petri dishes were cultured were on the medium After 28 days developing calli were subcultured or transferred to regeneration medium.

Regeneration of plants: 28 days calli obtained on MS medium were cultured on generation medium (Boi2Y) designed by Bingham *et al.* (1975) containing 2 g m^{-3} years extract. Calli were incubated at 27± 2°C at 16 h photoperiod. After 28 days calli with embryoids or without embryoids were subcultured onto fresh Boi2Y medium, which was necessary to promote conversion of embryoids and buds into plantlets and to enhance the growth of plantlets. Later, another subculturing was made depending upon the condition of the plantlets. At the end of second or third subculturing, plantlets mostly with roots or without roots were transplanted to jiffy-7 peat pots. To facilitate rooting, to prevent drying up, to give time to plantlets to acclimatize in vitro and become hardened off for *in vivo* climate, plantlets were covered for a period with transparent plastic covers. After two depending upon the health of the plantlet, plants were transferred to soil l and maintained in the Greenhouse.

Results and discussion

Screening of regenerator plants: 100 individual from each cultivar, of each species, using some explants

Table 1: Regeneration frequency and accomplishment of regenerated plants from different cultivars of *Medicago Sativa* and *M. media*

Species	Cultivar	Source of Explant	No. of Explant	R. Calli	Proper plantlets	%age regeneration
Medicago	Dupuits	Seedling	75	5	-	7
Sativa		Adult plant	25	2	-	
	Spreador-2	Seedling	75	8	4	14
		Adult plant	25	6	-	
	Roamer	Seedling	61	6	-	10
		Adult plant	39	4	1	
	Rangelander	Seedling	60	10	-	17
		Adult plant	40	7	1	
M. edicago	Rambler	Seedling	61	12	3	26
Mediaa		Adult plant	39	14	9	

R. Call=Regeneration calli; Seedling=Seedling explant (radicle, hypocotyl and cotyledon); Adult plant=Petiole explant

of petioles of parental (donor) plants grown in the Greenhouse and others from seedlings (radicles, hypocotyles and cotyledons) grown *in vitro* were used. The kind and number of explants used and their subsequent regenerative frequency and proper plantlets collected are shown in Table 1. To find out the regeneration capability of callus of single individual plants, calli were induced from different explants in case of seedlings i.e. radicles, hypocotyles and cotyledons and petiole from adult plants (Table 1). Cultivar Dupuits did not yield full plant regeneration, however, seven explants from different plants showed signs of regeneration, two with leaf like structures and five with only roots. On the other hand, four seedlings explant (radicles, hypocotyles and cotyledons) roots. On the other hand, four seedlings explant (radicles, hypocotyles and cotyledons) of cultivar Spreador-2 produced plantlets. A total of 12 plantlets were collected i.e. plant from each explant. After fourth week of growth on callus inducing medium calli were transferred onto Boi2Y medium. On transfer to Boi2Y medium these calli produced clear embryoids (Fig. 1a) which latterly developed into plantlets developed into plantlets, some times plants were collected from around the embryoids. In some cases plantlets developed from irregular deformed structures (Fig. 1b). Some calli produced only roots or hardened and rough leafy structures, which did not develop into proper plantlets. No regeneration was accomplished from seedlings explants of cultivars Roamer and Rangelander except deformed leafy structures and roots like that of Dupuits. However, cultivar Rangelander was more embryogenic than other cultivars of *M. sativa.* Explants from one adult plant of each cultivar fully regenerated. A total of four plantlets of Roamer and six plantlets of Rangelander were collected, respectively.

The Cultivar Rambler of *Medicago media* was highly embryogenic and showed the highest regeneration frequency and more fully developed plantlets (Fig. 1c). Three regenerators from seedlings and nine from adult plants were second out. Here, again it was observed that regenerator produced plantlets from each part of seedling used as explant (radicles, hypocotyles and cotyledons). The Parental plants were labeled and identified as regenerator. Regenerated plants were transplanted into jiffy-7 peat pots (Fig. 1d) after hardening off, these were transferred into soil pots and grown in the Greenhouse. To confirm and to find out whether regenerated plants retained the regeneration capability or they had lost it. The calli were produced from petioles of regenerators, which on transfer to regeneration medium showed response similar to their parental plants both in producing embryoids and plantlets.

This study illustrated that alfalfa plants can be regenerated from callus cultures. Similar results have been reported by Smith and McComb (1983) and Nagarajan *et al.* (1986) The percentage of regeneration is lower, however, it lies in the range reported by Bingham and McCov. (1986). In order of regeneration preference the "tested species and cultivars" could be ordered as *M. media* (Rambler) followed by *M. sativa* (Spreador-2, Rangelander, Roamer) and Dupuits. The collection of plantlets from radicle, hypocotyl and cotyledon of the same seedling revealed that plantlets can be obtained from all parts of regenerator plants.

Somaclonal variations: Somaclonal variations were assessed on their morphological appearance at the cellular and at the whole plant level. The whole plant level. The normal coloration of calli of all cultivars was whitish to pale-yellowish on incubation in the dark or in light (Fig. 2a). However, some of the replicates when incubated in the light synthesized chlorophyll (Fig. 2b) and red anthocyanin pigment (Fig. 2c). The nature, extent and possible origin of somalonal variations have been reviewed from various view points as chromosomal variation in cultured cells (Bayliss, 1980) and in regenerated plants (Lee and Phillps, 1988). Somaclonal variation results from both pre-existing genetic variation with in the explant and variation induced during the tissues culture phase (Evans *et al.,* 1984; Duncan, 1997). According to Lorz *et al.* (1988) plants regenerated via somatic embryogenesis are usually less variable than plants regenerated through organogenesis because the

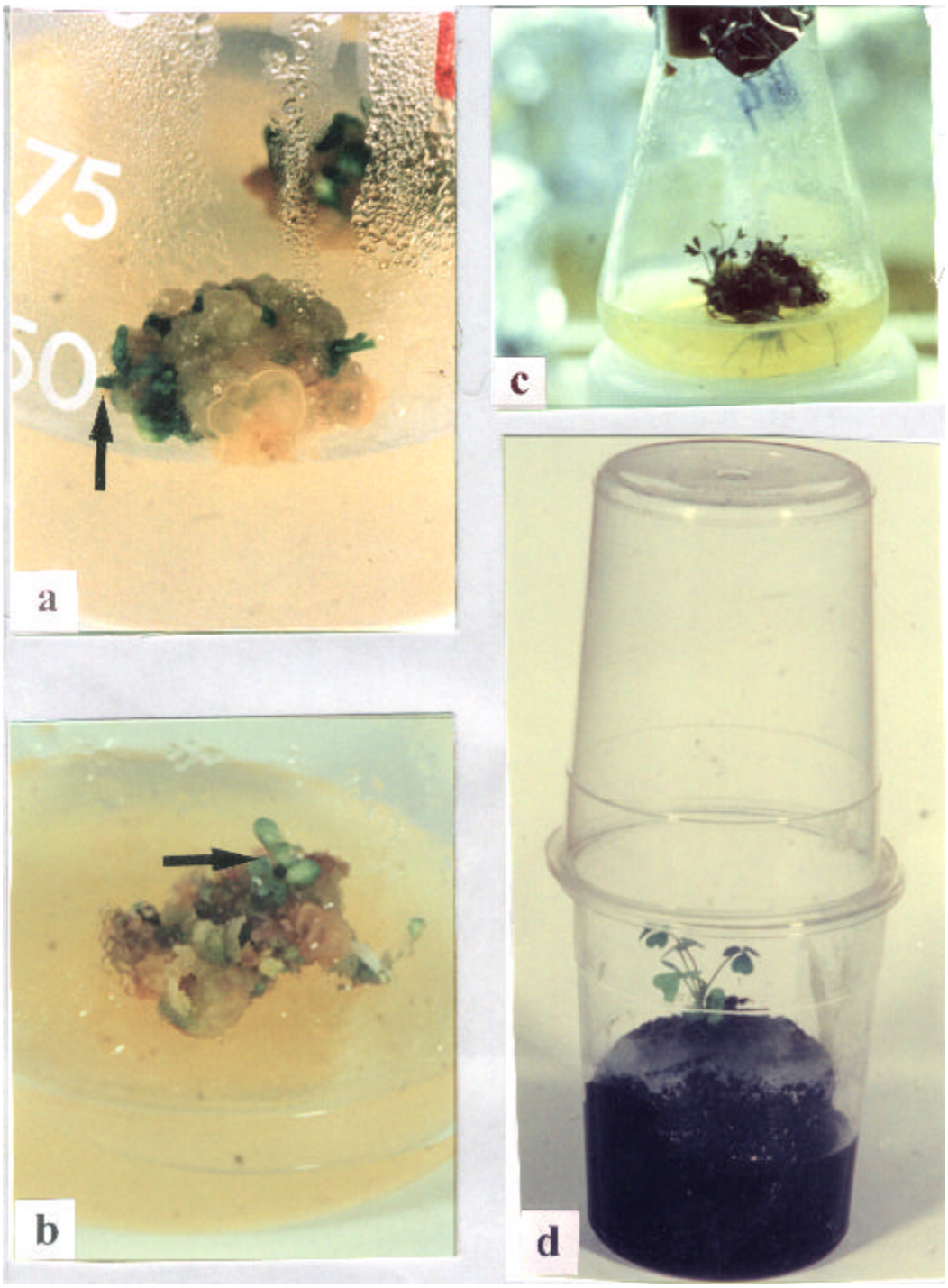

Fig. 1: a: Well-developed embryoids on semisolid medium; b. Deformed and irregular rough structure; c. Plantlets in the culture flask; d. normal healthy plant in jiffy peat.

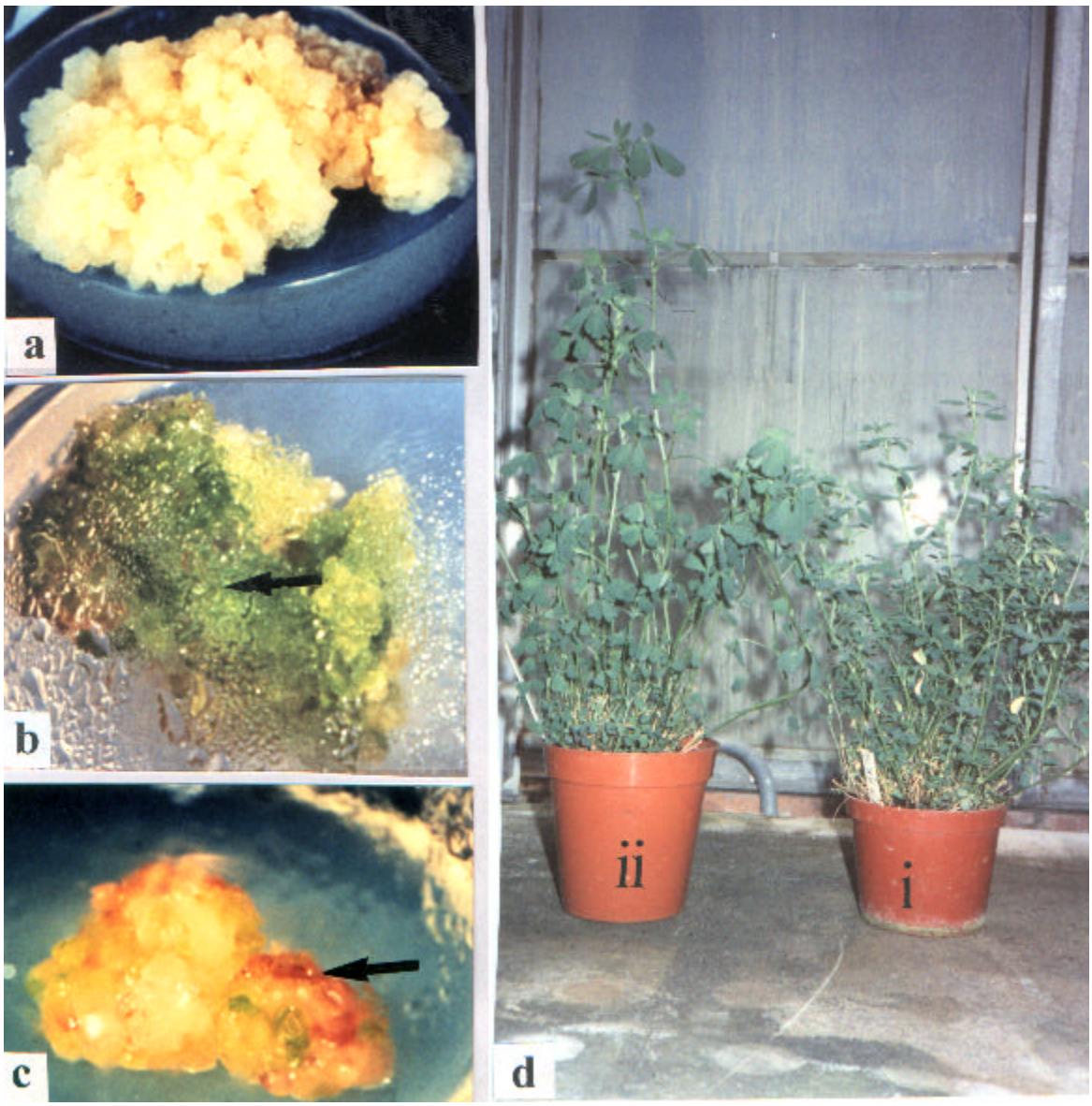

Fig. 2 a: Normal color of callus culture; b. A chlorophyll synthesizing variant; c. An anthocyanin synthesizing variant; d. (I). A normal plant from cv. Spreado-2 (ii) A somaclonal variant with large leaves, long internodes and vigorous growth from cv. Spreador-2

embryoid is generally derived from single of fewer cells than organogenesis. Bingham and McCov (1986) reported that not all somaclonal variation occurs variation occurs due to change in chromosome number but only 60% and about an equal percentage of somaclonal variation appears due to other reasons particularly gene expression as reported by Groose and Bingham (1986). They found that a white flowered mutant from a purple donor plant was due to switching off the anthocyanin gene during culture with loss of chromosome carrying the functional allele for synthesis. Upon reculturing the white flowered mutant 40% plants had normal flower color, which shows an unstable systems for anthocyanin synthesis.

Morphologically, almost regenerators looked similar to the normal donor plants except for one regenerator from cultivar Spreador-2. This plant showed vigorous growth, with large leaf size and long internodes Fig. 2d (ii). which was indicative of being polyploid. Bingham and Saunders (1974) reported that larger leaves in regenerated alfalfa plants had double chromosome number. This study

revealed that cultivar Rambler of *M. media* had higher regeneration potency than cultivars of *M. sativa* that high utility in biotechnology research targeting crop improvement for abiotic stresses.

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