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Cotyledon Cell Number and Size in Mature Seed and Seedlings of Birdsfoot Trefoil

by

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Broadleaf birdsfoot trefoil (*Lotus corniculatus* L.) has small seed compared to many other forage or grain legumes. Small seed size is one of the main factors responsible for poor seedling vigor, a major limitation of this important forage crop. Factors influencing seed size and cotyledon expansion during seedling growth have not been researched in birdsfoot trefoil. Two studies were conducted to determine: a) if variation in seed size was due to changes in cotyledon cell number and/or size, and b) which factors, cell division or cell enlargement, were responsible for cotyledon expansion in growing seedlings.

Studies on other legumes indicate that larger seeds can result from greater cotyledon cell number, larger cells, or both. Egli (1981) and Guldan and Brun (1985) suggested that much of the difference in seed size of soybeans (*Glycine max* L.) was related to the number of cells in the cotyledon. Swank et al. (1987) reported that larger seeds of soybean were associated with a larger number of cotyledon cells, but cell size also contributed to genotypic differences in seed size. Nimbkar et al. (1981) found that seed weight in peanut (*Arachis hypogaea* L.) was associated with cotyledon cell number more that cell weight. In contrast, Joshua and Bhatia (1983) found that larger seeds in peanut, pigeon pea (*Cajanus cajan* L.), and induced mutants of black gram (*Vigna mungo* L.) had increased cotyledon cell volume but not an increased number of cells. Both cotyledon cell number and weight were responsible for large seeds in pea (*Pisum sativum* L) (Davies,1975) and 12 species of the genus Vicia (Davies, 1977).

In species with epigeal emergence, such as birdsfoot trefoil, cotyledons can serve both as storage and assimilation organs. The capacity of the cotyledons as assimilation organs is associated with the initial size of the cotyledons and their ability to expand. Epigeal species vary in cotyledon expansion after emergence into the light (Lovell and Moore, 1970). In bean (*Phaseolus vulgaris* L.), cotyledons do not expand significantly, do not develop stomata, and lose weight rapidly during germination (Lovell and Moore, 1970; Opick, 1966). In lupin (*Lupinus albus* L.), cotyledons have moderate expansion, about doubling in size, and lose dry weight, although less rapidly than in bean. In species such as red clover (*Trifolium pratense* L.), gourd (*Cucurbita pepo* L.), mustard (*Sinapis alba* L.), and cucumber (*Cucumis sativus* L.)

cotyledons expand several times their origional size and increase in dry matter during early expansion (Lovell and Moore, 1970).

Hur, Beuselinck, and Nelson (1988, personal communication) found that birdsfoot trefoil cotyledons expanded several times after emergence and increased in dry weight. Maximum cotyledon expansion was reached at about three weeks after emergence. Larger seed had a larger initial cotyledon area and expanded more than those of small seed. Whether cotyledon expansion in birdsfoot trefoil is due to cell division or cell enlargement had not been researched.

The first study was conducted to determine if variation in seed size was due to changes in cotyledon cell number and/or cell size. Mature seeds of MU-81 and 'Norcen' birdsfoot trefoil were separated into three seed size classes. Cotyledons from each seed size class were macerated in chromic acid. Cell number was determined from aliquots of cell suspension. Cotyledon cell size was estimated by measuring cell area and by calculating the number of cells per mg of cotyledon dry mass and the dry mass of an individual cell. Cell number per seed increased 44% and 50% and cell area increased 47% and 34% from the small to large seed class in Norcen and Mu-81, respectively. Thus, larger seeds were associated with both and increase in cotyledon cell number and cell size.

A second study was conducted to determine which factors, cell division and or cell enlargement, were responsible for cotyledon expansion in growing seedlings. Seeds of MU-81 were germinated and grown in a growth chamber. Cell number and area of expanding cotyledons was measured every four d for 20 d. Methods similar to the first study were used except that cotyledons were pretreated with a dilute pectinase solution prior to maceration in chromic acid. Cotyledon expansion was associated with an increase in cell size but not in cell number. Thus, the maximum size an expanding cotyledon can attain was limited by the number of cotyledon cells in a mature seed and their ability to enlarge. Assuming a limit to the ability of individual cells to expand, cotyledon area is limited by the number of cotyledon cells in the mature seed. Selection for birdsfoot trefoil seeds with greater numbers of cotyledon cells may result in greater potential for cotyledon expansion. Seedlings with greater cotyledon expansion could be expected to have more vigorous seedling development.

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