Molecular Marker Identified Selfed Progeny and their Breeding Implications in Tetraploid Alfalfa Synthetics

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Alfalfa (*Medicago sativa* L.) is a major forage legume. Selfing (i.e. self-pollination) in alfalfa is possible, particularly in the absence of pollen from another genotype. Selfing has also been shown to occur in insect pollinated seed production fields. Reported selfing rates under field conditions range from 2% to 53%. By and large alfalfa breeders have ignored selfing in their plant breeding programs except in breeding schemes requiring intentional selfing. Even modest selfing rates will significantly increase inbreeding depression, particularly in successive synthetic seed increase generations. Inbreeding depression due to selfing dwarfs inbreeding effects associated with narrow versus broad-based synthetics. Prior to molecular markers, identifying selfed progeny in a standard alfalfa polycross or seed production field was almost impossible. In this study we present a SAS software program and simple DNA test utilizing SSR markers amplified in one or two PCR reactions that can identify selfed progeny in tetraploid polycrosses. The DNA test is demonstrated on a 15 parent autotetraploid alfalfa polycross pollinated with leafcutter bees (*Megachile rotundata* F.). In the demonstration polycross a 45% selfing rate was observed, consistent with previous studies. Outcross and self progeny were phenotyped for biomass yield and the synthetic's genetic load was estimated. Based on the synthetic's genetic load and selfing rate we estimated a 7% reduction in biomass yield from the syn 1 to syn 3 generation. One way to mitigate this yield loss would be to actively select for reduced selfing. A large range in selfing rates were observed between genotypes suggesting that selection for this trait should be feasible.