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Discovery and applications of double haploid inducing lines in rapeseed

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F009 (Brassica napus, 2n=38, AACC) was crossed with YH (Brassica campestris, 2n=20, AA), we obtained F1 polyploid using colchicine. In F3 generation, we found a tetraploid stable line (P3-2, 2n=38, AACC). P3-2 as female was crossed with two F1, (1325Cx, 2n≈58, AAAACC) and (4247X136, 2n=38, AACC) respectively, and we obtained polymorphic F1 hybrids. By the doubling of chromosome of F1, we obtained two polyploid 3380 and 3560. Cytological analysis revealed that 3380 and 3560 were octaploid (2n≈76, AAAACCCC). 3380 and 3560 were crossed as male with a Polima cytoplasmic male sterile line A0464 (2n=38, AACC) for test cross. All the F1 plants were surprisingly sterile and have same phenotypes to that of A0464. We obtained consistent results in repeated experiments, and found Y3380 and Y3560 were possible doubled haploid inducer.

In order to further verify the induction of Y3560, we crossed three nuclear male sterile lines 3209, 3304, and 3318 (Brassica napus, 2n=38, AACC) and four cytoplasmic male sterile lines 3321,3323,3445, and 3463 (Brassica napus, 2n=38, AACC) as female with Y3560. Results of all F1s were inducted successfully, with the induction rate of 34.09%~98.66%. Differential induction rate of Y3560 and Y3380 were found 95.15% and 93.33% respectively. From the evidences we concluded that Y3560 and Y3380 were not significantly different but can induce high rate of double haploid in rapeseed (Brassica napus, 2n=38, AACC).

Moreover, similar haploid induction behavior of Y3560 and Y3380 was found in Brassica juncia and Brassica campestris. But the induction lines were not stable, as we obtained tetraploid (10%) and hexaploid in the selfing generations. Tetraploid plants were unable to induce haploid induction. The cytological studies of Y3560 and Y3380 revealed severe abnormalities in meiosis such as presence of laggard chromosomes, and chromosomal bridge. The result of GISH revealed both induction lines were having some chromosome of A (larger proportion) and C genome. Interestingly, cross of Y3560 and Y3380 as male parent with the family of cruciferous vegetables (cabbage, radish, cauliflower, leaf mustard etc), F1 was successfully inducted and some of them displayed the appearance of vegetables. The molecular mechanism of induction of double haploids still needs further investigations.

Keywords: Chromosome doubling, Cruciferous vegetables, Double haploid inducer, Interspecific hybridization, Brassica napus L., Octoploid