Use of species-specific repetitive DNA probes in understanding the phylogenetic lineages of wild species belonging to Brassica-coenospecies

Brassica-coenospecies comprises a group of related taxa capable of artificial fertilization with Brassica crop species[1]. Extensive studies have been carried out to analyse the species relationships of crop brassicas and their wild relatives. Morphology[2,3], cytology, extent of hybrid fertility[1,4,5] and RFLP analyses of nuclear, chloroplast and mitochondrial DNA[6–11] have been used in these studies. On the basis of the RFLP analyses of nuclear and organelle DNA, some researchers have proposed two lineages in Brassica-coenospecies – *rapa/oleracea* lineage and *Sinapis/nigra* lineage[6,10,11].

Repetitive DNA is present in all higher organisms and forms a fairly large proportion of the genome[12,13]. It is not subjected to natural selection and evolves more rapidly than conserved and single copy sequences. Repetitive DNA is, therefore, ideal for analysing the species relationships and phylogenetic lineages. Some of these repeats have been reported to be species-specific. Two such repeats – pBNBH-35 (specific to *B. nigra*[14]) and pA2-78 (specific to *B. campestris*[15]) – have been used in the present investigation to discern lineages of three wild species of Brassica-coenospecies – *Erucastrum abyssinicum*, (Rich.) O. E. Schulz, *E. gallicum* (Willd) O. E. Schulz, and *Diplotaxis siifolia* G. Kunze.

Total genomic DNA was isolated from young leaves using the method described by Dellaporta *et al.*[16] and digested with *Hin*dIII of *Sau*3A I or *Bam*H I according to the manufacturer's instructions. The digested DNAs were electrophoresed on 1.5% agarose gels and were transferred on to nylon membranes (Hybond N^+, Amersham). The probes were labelled with α^{32}P dCTP using multiprime labelling kit (Amersham) according to Feinberg and Vogelstein[17]. Southern blots of *Hin*dIII and *Sau*3A I were hybridized with pA2-78, and the *Bam*H I blot was hybridized with pBNBH-35. Hybridization and washing were carried under high stringency conditions according to Lakshmikumaran *et al.*[18].

Tandem repeat of unit size 177 bp, characterized from *B. campestris* has been reported to be present in most of the crucifers[19]. However under high stringency conditions, the probe hybridizes only when there is a high degree of homology (> 90%). Previous studies[20] showed that *B. oleracea* and a wild species, *Diplotaxis erucoides*, exhibited high degree of sequence homology (98 and 96% respectively) with this repeat. These results are in agreement with the lineages based on RFLP by Song *et al.*[6]. Also, this repeat does not hybridize to the DNA of *B. nigra*, *B. tournefortii* and *Eruca sativa* belonging to *Sinapis/nigra* lineage. The above studies suggest the association of this repeat to species belonging to *rapa/oleracea* lineage. In the present study, the probe hybridized with DNAs of *E. gallicum* and *D. siifolia* under high stringency conditions giving multimeric bands of 177 bp, whereas *E. abyssinicum* DNA did not hybridize even under low stringency conditions. Thus the present results suggest that *E. gallicum* and *D. siifolia* are closely related to the species belonging to *rapa/oleracea* lineage.

A dispersed repetitive DNA (pBNBH-35) from *B. nigra* has been shown to be highly specific to *B. nigra* and does not hybridize to *B. campestris* or *B. oleracea* DNA. This repeat has been shown to hybridize with the digenomic Brassica species having *B. nigra* genome (BB) and a wild species *Sinapis arvensis*[14] belonging to *Sinapis/nigra* lineage. In the present study, under high stringency conditions of hybridization and washing, this probe hybridized only with *E. abyssinicum* DNA giving a specific band at 0.6 kb and not to that of *D. siifolia* and *E. gallicum*, suggesting that *E. abyssinicum* falls under *Sinapis/nigra* lineage.

On the basis of chloroplast DNA analyses, Warwick and Black[9] and Pradhan *et al.*[11] classified *E. abyssinicum* under *rapa/oleracea* lineage and *D. siifolia* under *Sinapis/nigra* lineage. The present study, however, assigns them vice versa. Our results as well as those of Warwick and Black[9] assign *E. gallicum* to *rapa/ oleracea* lineage. As reproductive isolation of the species belonging to Brassica-coenospecies is weak, the analyses based on chloroplast DNA are likely to be uniparentally biased because of exclusive maternal inheritance of plastids (see also Warwick and Black[9]). Repetitive nuclear DNA contributed by both the parents would be more useful in assessing phylogenetic lineages of the species belonging to Brassica-coenospecies.

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