Two distinct genetic diversity groups of oilseed *Brassica juncea* in both China and India

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ABSTRACT

Oilseed *Brassica juncea* is an agriculturally and economically important crop with a long history of cultivation in India and China, and increasingly in Australia. However, the centre of origin and diversity of oilseed *B. juncea* has been a controversial issue in the last century. In this study, allelic diversity of oilseed *B. juncea* predominantly from China and India was evaluated using simple sequence repeat (SSR) markers. Two major groups were identified and each included both Chinese and Indian accessions. Diversity groups 1 and 2 were also observed when A- and B-genome markers were analysed separately. The Shannon diversity index, based on analysis of geographical distribution, also revealed a diversity hotspot in central and western China. In this study, SSR allelic diversity in the A genome and B genome supported a polyphyletic origin and secondary centres of genetic diversity of oilseed *B. juncea* in China and India.

Key words: Indian mustard – microsatellite markers – private allele – allelic diversity –genetic diversity – genetic distinctness.

INTRODUCTION

Indian mustard (*Brassica juncea*) is an agriculturally important oilseed crop with a long history of cultivation in India and China, and increasingly in Australia. However, the centre of origin and diversity of oilseed *B. juncea* has been a controversial issue in the last century.

B. juncea (2*n* = 36, AABB) is believed to be an amphidiploid derived from interspecific hybridisation between *B. rapa* (2*n* = 20, AA) and *B. nigra* (2*n* = 16, BB) (U, 1935). Several researchers held the view that *B. juncea* originated at multiple places in the Middle East and neighbouring regions where wild *B. rapa* and *B. nigra* overlapped in their distribution (Burkill, 1930; Olsson, 1960; Vaughan *et al*. 1963), whereas others considered that several independent polyploidisation events occurred to evolve into present-day agricultural types of *B. juncea* (Prakash and Hinata, 1980). Prain (1898) proposed that the common oilseed mustard Rai was an immigrant into India from China through a north-eastern route. This proposition was supported by Sinskaja (1928) but disputed by Burkill (1930) and Sun (1970). Vavilov (1949) proposed that Afghanistan and adjoining regions was the primary centre of origin of *B. juncea*, and considered Asian Minor, central/western China and eastern India as secondary centres of diversity. Vaughan *et al.* (1963) and Vaughan (1977) proposed two geographical races of oilseed *B. juncea*: the Indian race and the Oriental (i.e. Chinese) race, and suggested that the Indian race was closer to the *B. rapa* progenitor, and the Oriental race was closer to the *B. nigra* progenitor. However, Indian researchers considered that Indian *B. juncea* forms might have originated in India itself (Duhoon and Koppar 1998).

Molecular markers provided an important tool for the genetic diversity study of Indian and Chinese oilseed mustard. All Indian accessions grouped together as assayed by random amplified polymorphic DNA (RAPD) (Jain *et al.*, 1994; Khan *et al.* 2008). As assayed by amplified fragment length polymorphism (AFLP) markers, all the Indian and Chinese *B. juncea* lines were clustered together whereas lines from other sources formed another group (Srivastava *et al.* 2001). RAPD analysis indicated that the genetic relationship of Chinese oilseed mustard accessions was mainly decided by the specific ecological environment as well as the local cultivation customs and high diversity of *B. juncea* was discovered in south-western and western China (Pu *et al.* 2007). This is supported by the analysis using AFLP, sequence-related amplified polymorphism (SRAP) and simple sequence repeat (SSR) markers (Xu *et al.* 2008). Analysis using SRAP markers revealed that the Chinese oilseed mustards were divided into different groups mainly according to their growth habitat (Wu *et al*. 2009).

Since *B. juncea* is an allotetraploid species containing A and B genomes from progenitor species *B. rapa* and *B. nigra*, knowledge of molecular genetic diversity and distinctness in the A and B genomes of present-day oilseed *B. juncea* would help to resolve the question of the geographic origin and genetic diversity of Indian and Chinese oilseed *B. juncea*. In this study, we used locus-specific simple sequence repeat (SSR) markers covering the A and B genomes to genotype a collection of oilseed *B. juncea* landraces, cultivars and breeding lines from China and India, as well as cultivars and breeding lines from Australia and Europe. The objective of this research was to dissect genetic diversity in the A and B genomes, and to shed light on the origin and evolution of oilseed *B. juncea* in India and China.

MATERIALS AND METHODS

A total of 123 *B. juncea* accessions (landraces, cultivars and breeding lines), including 53 from China, 46 from India, as well as 12 from Australia and 12 from Europe, were collected for this study. Samples from China and India were chosen from the accessible germplasm sources to cover the widest possible geographical distribution and the longest possible breeding history.

A total of 99 SSR primer pairs were used for the genetic diversity study, with 51 and 48 primer pairs to target the A and B genomes of *B. juncea*, respectively. The primer sequences were kindly provided by A. Sharpe and D. Lydiate (Agriculture and AgriFood Canada Saskatoon Research Centre, Saskatoon; pers. comm.; for more information, see http://brassica.agr.gc.ca).

DNA extraction, PCR procedures, and multivariate analysis were performed following Chen *et al.* (2010). The allelic diversity across all *B. juncea* accessions was assessed for common and private SSR alleles in populations from Australia (AU), China (CN), Europe (EU) and India (IN) using the method described by Chen *et al.* (2008; 2010). Common alleles were shared between two or more countries; private alleles were unique to one country.

Geographical distribution of allelic diversity of Chinese and Indian *B. juncea* accessions was analysed according to the longitude and latitude of the capital city of each State or Province where the accession was collected. Shannon diversity index was calculated using GenAlEx ver 6.3 (http://www.anu.edu.au/BoZo/GenAlEx/) based on the paper by Sherwin *et al.* (2006).

RESULTS AND DISCUSSION

Allelic diversity in the A and B genomes of oilseed *B. juncea* was, for the first time, evaluated using SSR markers covering the A and B genomes in a large collection of landraces, cultivars and breeding lines from India, China, Europe and Australia. Five hundred polymorphic alleles from both A and B genomes divided 119 oilseed *B. juncea* accessions from Australia (AU), China (CN), Europe (EU) and India (IN) into 2 major groups at the genetic dissimilarity level of 0.47: Groups 1 and 2 both contained Indian and Chinese *B. juncea* accessions, while European and Australian accessions were found only in Group 2 (Fig. 1). Allocation of accessions to Groups was consistent across the A and B genomes, indicating that *B. juncea* had similar patterns of genetic dissimilarity in both A and B genomes.

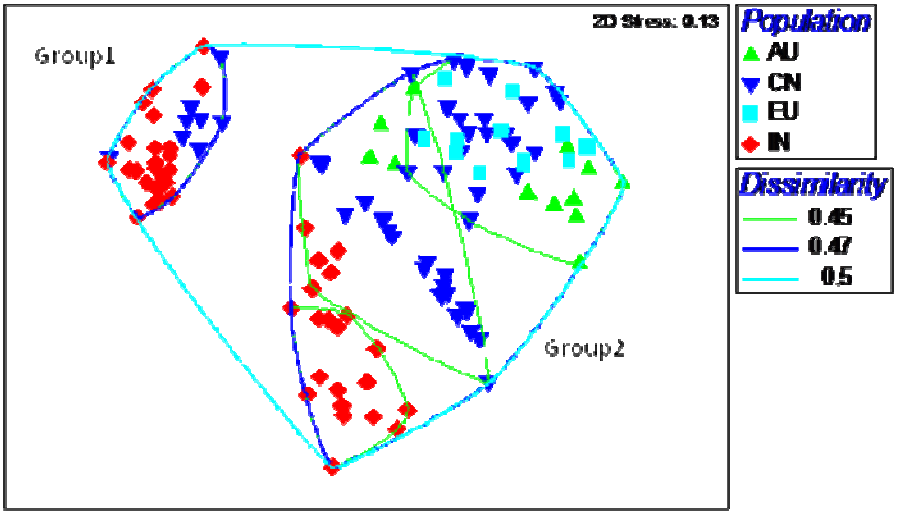

Fig. 1. Five hundredpolymorphic alleles from both A and B genomes divided 119 oilseed *B. juncea* accessions from Australia (AU), China (CN), Europe (EU) and India (IN) into two major groups at the genetic dissimilarity level of 0.47: one small group (Group 1) with 8 CN accessions and 23 IN accessions, and one large group (Group 2) with the remaining 88 *B. juncea* accessions.

The Shannon diversity index, based on analysis of geographical distribution, revealed two high diversity regions: one was based in West Bengal in eastern India with the diversity index as high as 0.264 and the other was in central and western China with the diversity index of 0.224. Due to the limited number of accessions from West Bengal, the diversity hotspot there is uncertain. However, the diversity hotspot of *B. juncea* around Yunnan, Qinghai and Gansu in China is clear.

Our results support Vavilov's (1949) hypothesis that there was a primary centre of origin of *B. juncea* in central Asia, and that China and India are secondary centres of diversity. Our data also support the new proposition that there were two independent migrations of *B. juncea* into China and India, which are revealed today as diversity groups 1 and 2. Group 1 and Group 2 would be two independent migrations to China and India. n this study, SSR allelic diversity in the A genome and B genome supported a polyphyletic origin and secondary centres of genetic diversity of oilseed *B. juncea* in China and India.

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