Fagopyrum Species Cultivated in Himalayan Regions

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Fagopyrum spp. (buckwheat) is a dicotyledonous pseudocereal crop mainly cultivated in the north-western Himalayan regions for its highly nutritional, antioxidant and therapeutic values.

buckwheat genetic diversity ISSR markers

1. Introduction

Fagopyrum spp. (buckwheat) is a minor pseudocereal food crop from the family of *Polygonaceae* consisting of about 26 species (wild and cultivated) known so far [1][2][3]. Its grains are enriched with flavonoids, especially rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) and some vitamins (B1: thiamine; B2: riboflavin; B3: niacin; B6: pyridoxine; C: ascorbic acid and E: tocopherol). The biological value (BV) is higher due to presence of essential amino acids, particularly lysine, valine, tryptophan, arginine, threonine, isoleucine, leucine, phenylalanine and sulfur-containing amino acids such as cysteine and methionine [4][5][6]. The flowers of buckwheat during blossoming are enriched with a high quantity of flavonoids, especially rutin or sophorin which is known for anticancer, anti-mutagenic, antioxidant, antidiabetic, anti-inflammatory, anti-bacterial, anti-fungal, neuroprotective, cardioprotective, hepato-protective, nephron-protective, haemato-protective, anti-arthritis and anthelminthic properties [7][8][9][10][11]. Consequently, it has immense value in the functional food industry due to its unique nutritional status and considerable nutraceutical properties, which makes it a smart candidate for future crops. However, it is a minor crop and received little attention so far, especially in developing nations where it holds a great diversity in terms of species diversity, growth pattern and development. Despite having considerable genetic variation in the *Fagopyrum* genus, no significant contribution has been made in the development of improved cultivars. To address this problem, there is an urgent need to develop global databases and core collections of accessions available in the seed/genebanks for organized evaluation to decipher the enormous genetic ability of buckwheat germplasm. Although, due to inter-specific crossing between genetically distant buckwheat species, small achievements have been made, more efforts are needed to overcome certain barriers. Moreover, inter-specific crossing between wild and cultivated forms of buckwheat can help in the transfer of some of the desirable agronomic traits in the latter. For instance, the wild relatives of *F. cymosum*, *F. urophyllum* and *F. gracilipes* consist of a high content of amino acids, proteins, phenols and flavonoids in comparison to cultivated species (*F. esculentum* and *F. tataricum*), and these characteristics (genetic variations) could be utilized for crop improvement programmes of buckwheat [12].

2. History and Development

Buckwheat has been first reported to be grown in China around 1000 BC, and from here it was introduced to other parts of the world [13][14]. It is still being cultivated throughout the world, especially in high altitude mountainous regions possessing diverse topography. This is of great advantage as it conserves the diversity in the germplasm of different buckwheat species. The assessment of genetic diversity is an important aspect of any program aimed at improving crops. Relationships and variability within and among the accessions/landraces of buckwheat species and their population structure have been elucidated by using a wide range of molecular markers, viz., allozymes [15][16], RFLP (restriction fragment length polymorphism) [17], ITS (internal transcribed spacer) and the conserved sequences based on mitochondrial and chloroplast DNA [18][19][20], RAPD (random amplified polymorphic DNA) [21][22][23], AFLP (amplified fragment length polymorphism) [24][25][26], SSR (simple sequence repeats) [27][28], SRAP (sequence-related amplified polymorphism) [29] and STS (sequence-tagged site) [30]. The markers based on ISSRs are quite efficient for determining genetic diversity at low levels. It is a promising DNA based dominant marker system for studying genetic diversity, population genetics, gene tagging, genome mapping, phylogeny and evolutionary biology in many plant species [31]. In some ways, the ISSR marker technique has alleviated the limitations of RAPD (low reproducibility), AFLP (high cost) and creates species-specific primers for SSR markers [32]. ISSR is an arbitrarily primed PCR-based technique first reported by Zietkiewicz et al. [33] in which the primers amplify the sequences between two microsatellite regions, producing a multi-locus marker system that is useful for the identification of species, genotypes, cultivars or populations and for evaluating genetic variation at intra-and inter-specific level [34][35]. It involves primers that are based on repeat sequences along with a selective nucleotide degenerate 3′ anchor, such as $(CA)_6$ RG or $(ACG)_8$TY. Furthermore, ISSR markers use longer primers, usually 16–25 mer, as opposed to 10 mer primers in RAPD, which allows higher annealing temperatures and, thus, improves reproducibility [32][33].

There is great significance with respect to inter-specific hybridization among *Fagopyrum* species as it allows the transfer of agronomically important traits such as cold tolerance, enhanced fertility, low seed shattering, increased content of rutin and other neutraceutically important compounds from domesticated and wild relatives of *Fagopyrum* species. Furthermore, genome mapping and conventional breeding are the essential biotechnological tools that are important in the crop improvement of buckwheat germplasm through the identification and transfer of important genetic traits within and among the species [36][37]. Studies based on various types of molecular markers in sweet buckwheat (*F. esculentum* Moench) revealed that its local populations are actually represented as highly heterozygous and heterogeneous. Moreover, it has also been reported that certain South-European landraces exhibit a lower amount of genetic variation when compared to Asian and North-European landraces in addition to greater genetic differentiation that can even be visualized as variations in seed groat colour [38].

3. Distinct Cultivated Species

The genus *Fagopyrum* is represented in Kashmir and Ladakh regions by four distinct cultivated species consisting of sweet (*F. esculentum* Moench), bitter (*F. tataricum* Gilib), coarse (*F. sagittatum* Gaertn) and kashmir (*F.*

kashmirianum Munshi) buckwheats [39][40]. Although in contrast to the mixed cropping used in other areas of India, buckwheat is more commonly grown as a healthy crop in the north-western Himalayan region. It has been recently reported that buckwheat species grown in these regions are enriched with significant concentrations of phenolic and flavonoid compounds, in addition to possessing higher antioxidant potential and essential macro-and micronutrients revealing their therapeutic value and, therefore, can be considered as a potential crop for bio-fortification [41]. In light of the above, this study used ISSR markers to analyse intra-specific and inter-specific variability as well as population structure of four buckwheat species grown in these regions.

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