Exploration, Collection and Characterization of Kala zeera (Bunium persicum Boiss. Fedtsch.) Germplasm from North-Western Himalayas

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Abstract

Kala zeera (*Bunium persicum* Boiss. Fedtsch.) is one of the most important spice crop in the world. A set of two hundred fifty two (252) diverse kala zeera germplasm accessions were collected during an exploration mission from hot-spot regions /hills dividing two neighboring countries (India-Pakistan) on line of control (LOC) and hills near line of actual control (LAC) with China. The crop grows wild in its natural habitat mainly in Gurez valley, Tulail, Kashtiwar, Keran, Machil Tangdhar, Machil, Drass, Paddar, Khrew, Char-e-Sharief, Pang, Lahaul spiti, Shaung, Bharmour and Almora hills of Indian Western Himalayas. The germplasm collected has been characterized for morpho-agronomic traits and the analysis of trait data revealed significant variability in number of branches plant$^{-1}$, number of umbelets umbel$^{-1}$, number of seeds plant$^{-1}$, seed yield per plant and 1000 seed weight. The collection and characterization of 252 Kala zeera germplasm accessions can prove useful in future Kala zeera improvement programs in the world as this is first such comprehensive report of the crop from Western Himalayan region of India.

Introduction

Kala zeera (*Bunium persicum* Boiss. Fedtsch.) is an economically important medicinal spice, condiment as well as aromatic plant belonging to family *Apiaceae*. It is perennial, glabrous, branched herb native to Europe and Western Asia and has been found growing in dry temperate areas in the Western Himalayan region of the Indian sub-continent including Gurez valley at an altitude between 2000–3000 m above m.s.l. and extending up to Baluchistan and Afghanistan (Bhartiya, 1967). High altitude regions of Gurez valley, Kashtiwar, Keran, Machil Tangdhar, Machil Paddar, Khrew, Char-e-Sharief, Drass and Harwan in Jammu and Kashmir, Lahaul spiti, Shuang, Pang, Bharmour in Himachal Pradesh and Almora hills of Uttarakhand states are important hot-spot areas of its production in India (Panwar *et al.* 1993). As per the latest figures of state forest department of Jammu and Kashmir for this crop, average productivity of kala zeera in the cultivated areas is around 129 kg ha$^{-1}$.

Among the important kala zeera growing areas of north-western Himalayas, Gurez located on Indo-Pakistan border, is an important kala zeera growing area in the Jammu and Kashmir. It harbours various sub-populations within its forest pockets possessing significant genetic variability for this crop. This genetic variability has been observed in the form of land races that exist on other higher reaches of these western Himalayan states of India. In the recent past continuous unscientific and ruthless exploitation of this crop by locals of these areas for immediate financial gains has led to low productivity and genetic erosion of this crop. The drastic reduction in area under the crop has further aggravated the situation. Although some efforts for conservation of this crop were made earlier by different agencies, but their domain of work or resources were limited to establish an impact. The continued practice of unsustainable and unscientific exploitation of this crop species has resulted in reduction of its natural populations and has made it a plant of special conservation concern in these north-western Himalayan hills. If this situation is not taken care of, the zeera crop area may soon become drastically reduced which
may, ultimately, lead to heavy economic losses or extinction of this important seed spice. By far, the forest areas of Gurez, Tulail, Kishtiwar, Keran, Machil Tangdhar, Kargil, Paddar, Khrew, Char-e-Sharief, Drass, Kinnaur, Lahaul spiti, Pang, Bharmour, Shaung and Almora hills contribute maximum to the zeera production in north-western region and as such judicious domestication of this crop in these area for commercial cultivation is expected to contribute significantly. Further the efficient utilization of the genetic resources available in this crop species in these western Himalayan states of India for breeding through establishment of a kala zeera germplasm bank can contribute in enhancement of its production and productivity in the area. The study shall be the first such comprehensive report of Kala zeera accessions from Western Himalayan region of India.

**Exploration And Collection**

Keeping in view the importance of this crop and by keeping in view the fact that farmers are adopting unscientific practices that may result in erosion of this crop, efforts have been made to systematically collect landraces from their wild habitat in western Himalayas. All the hot-spot regions have been explored and efforts have been made to collect all existing diversity available in kala zeera crop. In summary, the exploratory survey route covered around 3000 km in northern states of Himachal Pradesh, Uttarakhand and Union Tertiary of Jammu and Kashmir (see Figure 1 for details), while the village-wise collection of kala zeera accessions is presented as Figure 2. The altitude of collection sites explored during the present study varied from 1524 to 4883 meters above mean sea level (Table 1). In all, 1000 samples, each of 1-2 tubers, were collected from tribal farmers or directly from natural habitat. Each zeera growing village was surveyed for identification of genetic variability and tagging of spots during months of June and July. The initial level of character variation was documented along with the spot number with area as reference data. With the help of local implementing agencies and tribal farmers kala zeera tubers were collected from these areas entry-wise during year 2019 and 2020 for their establishment at Mountain Agriculture Research and Extension Station (MAR&ES), Gurez, SKUAST-Kashmir.

The entire set of collection of landraces were initially evaluated during the months of May, June and July, 2020 at MAR&ES, SKUAST-Kashmir Gurez for establishing their genetic variability through morphological characterization. The diverse genetic stocks of kala zeera are expected to establish a Kala zeera Germplasm Bank at MAR&ES, SKUAST-Kashmir, Gurez. The seed from each selected germplasm accession will be used for molecular characterization studies of kala zeera germplasm, inter-relationship of landraces and to investigate evolutionary hierarchy of Kala zeera landraces.

**Characterization**

The survey and exploration visits of Kala zeera growing areas were conducted and each sample was collected either directly from natural habitat or from the local tribal farmers. Out of these kala zeera collections, around one thousand collections were planted after applying recommended doses of manures and planted at a spacing of 20 × 20 cm on scientific lines in the form of a germplasm bank at Mountain Agriculture Research Station, SKUAST-Kashmir Gurez (Longitude- 34°39’19.822°N, Latitude-
Out of these two hundred fifty two diverse kala zeera accessions have been characterized for morpho-agronomic traits at different growth stages.

Morpho-agronomic characters: The data on minimum, maximum, mean values, and coefficient of variability (CV) for eleven morpho-agronomic traits (days to flowering, days to 50% flowering, days to full bloom, plant height, number of branches plant\(^{-1}\), number of umbellets umbel\(^{-1}\), umbel diameter, days to 80% maturity, number of seeds plant\(^{-1}\), seed yield plant\(^{-1}\) and 1000 seed weight) involving 252 genotypes are presented in Table 2. Analysis results clearly showed significant variation in all these traits. The coefficient of variation (CV) values ranged from 3\% to 44.32\%. The morphological traits of Kala zeera showed a typical normal distribution. The highest mean value was observed for number of seeds plant\(^{-1}\) (110.85) followed by days to 80% maturity (89.66). The highest CV was found for seed yield per plant (58.53) followed by number of seeds plant\(^{-1}\) (49.84). However, lowest CV (3) was observed for days to 80% maturity.

Existence of genotypic variability for all the traits was further confirmed by PCA analysis (Table 3) that grouped the genotypes into four components. The different morphological traits contribute for total variation calculated for each component. The Component 1 has the major contribution of days to flowering (0.455), days to 50% flowering (0.518), days to full bloom (0.501), days to 80% maturity (0.481) to the total variability. The Component 2 has the major contribution of number of branches plant\(^{-1}\) (0.492), number of umbels plant\(^{-1}\) (0.503), number of seeds per plant (0.530) and seed yield per plant (0.349) to the total variability. Similarly, plant height (0.586) and number of umbelets umbel\(^{-1}\) (0.658) and plant height (0.584) and seed yield per plant (0.449) has contributed to total variation from component 3 and component 4, respectively.

Discussion

The exploration visits of kala zeera growing areas were conducted during years 2019 and 2020. These areas in Jammu and Kashmir mostly constitute difficult hills and terrains on Indian side of Indo-Pakistan border that are either restricted or often remain influenced due to cross border firing. In Himachal Pradesh and Uttarakhand also, kala zeera growing areas are high hills or terrains adjacent to border with China. During the present survey distinct morpho-agronomic characters were recorded for two hundred fifty two kala zeera collections after exploring different kala zeera growing areas of Jammu and Kashmir and states of Himachal Pradesh and Uttarakhand. The study of flowering and maturity traits among zeera collections revealed that the days to flower initiation ranged from 26 to 42 days, with the 24 genotypes identified as earliest (26 days) flowering types. Full bloom started from forty eight days after germination and was completed in all the entries till fifty ninth days. The genotypes took 83 to 94 days for attaining 80% maturity. High genetic variability of kala zeera collections was also reported by Mittal et al (2006) and Majeed et al (2008). Crop maturity is an important trait, earlier the genotype more is the possibility to vacate the field early for raising next crop. Under Gurez conditions, early maturing genotypes are expected.
to minimize the shuttering loss as they escape the speedy winds that start blowing from 20th of July each year.

The study of yield traits revealed that the highest per plant zeera yield were recorded by two entries from Nayaal and Chuntiwari areas of Gurez valley of Jammu and Kashmir (J&K) viz., KZG112 (6.4 g) and KZG215 (5.0 g) respectively. A Kala zeera entry from village Chorwan, Gurez viz., KZG192 and entry KZG255 from Shaung village Himachal Pradesh each recorded seed yield of 4.0 g per plant, whereas, 3g seed yield per plant was harvested each from entry KZG272 collected from Dras (J&K) and entry KZG208 collected from Chorwan, Gurez (J&K). Kala zeera entries recording high yields under domestication could be tested further for their possible exploitation as Kala zeera varieties and their use in future breeding programmes. Efforts could be made to register the promising entries with respect to earliness or yield in national germplasm repository, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

In summary, the present study revealed significant diversity/genetic variation available in Kala zeera germplasm in Western-Himalayas of India and therefore, offers very good scope for its improvement. The most promising germplasm accessions identified on the basis of trait data will be used in development of high yielding improved Kala zeera varieties for Western Himalayas. Some of the promising/candidate lines will also act as useful genetic resource for variety of genomics studies including transcriptomics for identification of differentially expressing genes and in mapping genes for important traits through development of bi-parental mapping populations. This is such first comprehensive study in Kala zeera from Western Himalayan region of India. Such efforts made will also help to conserve useful genetic diversity of Kala zeera in National/local gene banks for their further redistribution among breeders/farmers world-wide.

Declarations

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Code availability: Not applicable

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Tables

Table 1. Map location of sites for collection of Kala zeera germplasm accessions
| Area          | Longitude       | Latitude       | Altitude (m) |
|--------------|-----------------|----------------|--------------|
| Gurez        | 34.6494°N       | 74.7366°E      | 2,580        |
| Tulail       | 34.5559°N       | 75.0544°E      | 2,750        |
| Kishtiwari   | 33.3116°N       | 75.7662°E      | 1,638        |
| Keran        | 34.6651°N       | 73.961°E       | 1,524        |
| Machil       | 34.6923°N       | 74.3592°E      | 2,450        |
| Tangdhar     | 34.3975°N       | 73.8607°E      | 1,929        |
| Kargil       | 34.5539°N       | 76.1349°E      | 2,676        |
| Paddar       | 33.2658°N       | 76.1581°E      | 2,958        |
| Khrew        | 34.0209°N       | 74.9998°E      | 1,607        |
| Char-e-sharief | 33.8629°N   | 74.7663°E      | 1,933        |
| Drass        | 34.4306°N       | 75.7515°E      | 3,300        |
| Kinnaur      | 31.6510°N       | 78.4752°E      | 4,075        |
| Lahaul Spiti | 32.6192°N       | 77.3784°E      | 4,883        |
| Pang         | 30.0409°N       | 78.8652°E      | 4,600        |
| Bharmour     | 32.4411°N       | 76.5357°E      | 2,121        |
| Shong/ Shaung | 31.1100°N     | 77.1600°E      | 2,662        |

Table 2. Range, mean, standard deviation and coefficient of variation (CV) for growth and yield contributing traits
| Variable                        | Min | Max | Mean  | Std. Dev | CV%  |
|--------------------------------|-----|-----|-------|----------|------|
| Days to flowering              | 26  | 42  | 29.16 | 2.35     | 8.05 |
| Days to 50% flowering          | 36  | 48  | 39.77 | 2.62     | 6.58 |
| Days to full bloom             | 48  | 59  | 54.72 | 2.52     | 4.60 |
| Plant height                   | 12  | 67  | 35.54 | 9.66     | 27.18|
| Number of branches plant \(^1\) | 1   | 6   | 2.82  | 1.25     | 44.32|
| Number of umbelets umbel \(^1\) | 7   | 15  | 11    | 1.61     | 14.63|
| Umbel diameter                 | 8.1 | 11.9| 10.3  | 1.05     | 10.19|
| Days to 80% maturity           | 83  | 94  | 89.66 | 2.69     | 3.00 |
| Number of seeds plant \(^1\)   | 17  | 347 | 110.85| 55.25    | 49.84|
| Seed yield per plant           | 0.4 | 6.4 | 1.64  | 0.96     | 58.53|
| 1000 seed weight               | 1.56| 2.12| 1.9   | 0.11     | 5.78 |

Table 3. Principal component analysis (PCA) of growth and yield contributing traits

| Variable                        | PC1  | PC2  | PC3  | PC4  |
|--------------------------------|------|------|------|------|
| Days to flowering              | 0.455| -0.072| -0.079| 0.031|
| Days to 50% flowering          | 0.518| -0.101| -0.049| 0.056|
| Days to full bloom             | 0.501| -0.102| -0.024| 0.066|
| Plant height                   | 0.025| -0.021| 0.586 | 0.584|
| Number of branches plant \(^1\) | 0.084| 0.492 | -0.08  | -0.028|
| Number of Umbels per plant     | 0.147| 0.503 | 0.034  | 0.013|
| Number of umbelets umbel \(^1\) | 0.015| 0.144 | 0.658  | -0.193|
| Umbel diameter                 | 0.061| 0.178 | 0.297  | -0.604|
| Days to 80% maturity           | 0.481| -0.064| 0.053  | -0.109|
| Number of seeds plant \(^1\)   | 0.094| 0.530 | -0.046 | 0.057|
| Seed yield per plant           | -0.018| 0.349 | -0.166 | 0.449|
| 1000 seed weight               | -0.027| 0.139 | -0.294 | -0.175|

Figures
The collection and maintenance of Kala zeera germplasm in Western-Himalayas. The figure shows the map of India (a), Jammu and Kashmir (b), hot-spot regions/collection sites along indo-Pakistan border (c) GPS location of experimentation/maintenance site (d), and map location of the site of experimentation/maintenance of germplasm/establishment of germplasm bank (e). Note: The designations employed and the presentation of the material on this map do not imply the expression of
any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Exploration visits and germplasm accessions collected from different hot-spots/villages in North-western Himalayas. The figure shows details of number of germplasm accessions collected from different
Villages in north-western Himalayas of India (a), germplasm establishment site at Gurez (b), Collection site at Chorwan, Gurez (c), Collection site in Nyle-Valley, Gurez (d), and a Kala zeera plant at flowering stage in germplasm bank at Gurez on Indo-Pakistan border (e).

Figure 3

Variation available for morphological traits in Kala zeera collected from North-western Himalayas of India. The histograms shows the kind of distribution shown by growth trait data (late 1 to 5) and yield.
and yield attributing trait data (plate 6 to 10).