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SKUAST-Kashmir

Diversity, Value Addition and Marketing of Kala Zeera




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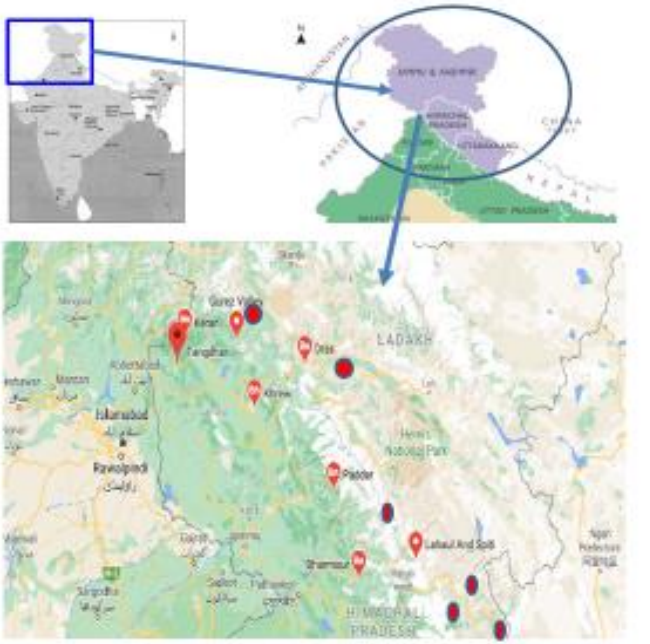
GERMPLASM COLLECTION AND CROP DIVERSITY

The area under kala zeera has reduced drastically particularly, since last few decades, in view of the fact that inhabitants of nearby areas usually go for harvesting of immature zeera. Kala zeera seed when harvested before maturity can be used as spice, but such zeera seeds are unable to germinate. This has contributed not only to low productivity but also lowering the genetic base of this crop (Khan *et al.,* 2021). The genetic diversity of the crop represents the heritable variation that exists within and between populations. Exploration and evaluation of genetic diversity is a prerequisite for identifying the suitable genotypes, which can be utilized further for initiation of a breeding programmes. The long term breeding programmes can result in development of elite genetic stocks for commercial cultivation. The success of a plant breeding programme depends on the availability of genetic variation, knowledge about desired traits and efficient selection strategies that makes it possible to exploit existing genetic resource. In spite of being a prized herb with many therapeutic uses, the information on genetic diversity present among the different populations of *B. persicum*, especially in North-western Himalayan region is severely lacking. Urgent and timely efforts are required to be taken for

conservation of genetic diversity and identification of kala zeera genotypes with high yield potential.

Under NMHS funded research project on Kala Zeera, a set of two hundred fifty two (252) diverse kala zeera germplasm accessions were collected during an exploration mission (figure 1 & 2) 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 (figure 3 & 4) 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 shall be 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.



a. Survey routes of exploration and collection of Kala zeera accessions

b. GPS Location of Site of Experimentation

c. Map Location of Site of Experimentation

Figure 1. Survey route of exploration and collection (a), site of characterization of Kala zeera accessions (b, c)

Source: Khan *et al* (2021) Plant Genetic Resource

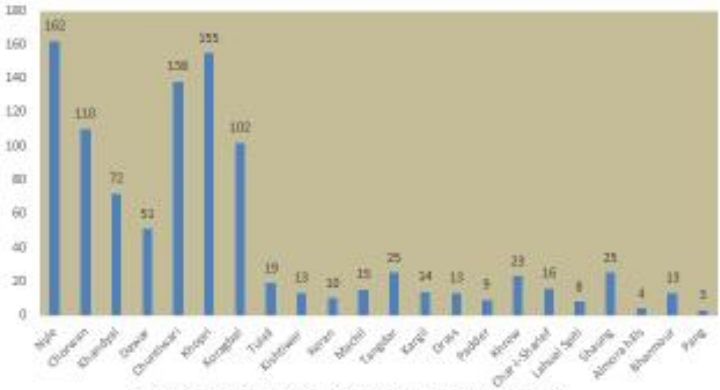


Plate2a. Village-wise collection of Kala zeera accessions

Plate2b. Exploration for collection of Kala zeera accessions

Figure 2. Exploration and collection of Kala zeera accessions

Source: Khan *et al* (2021) Plant Genetic Resource

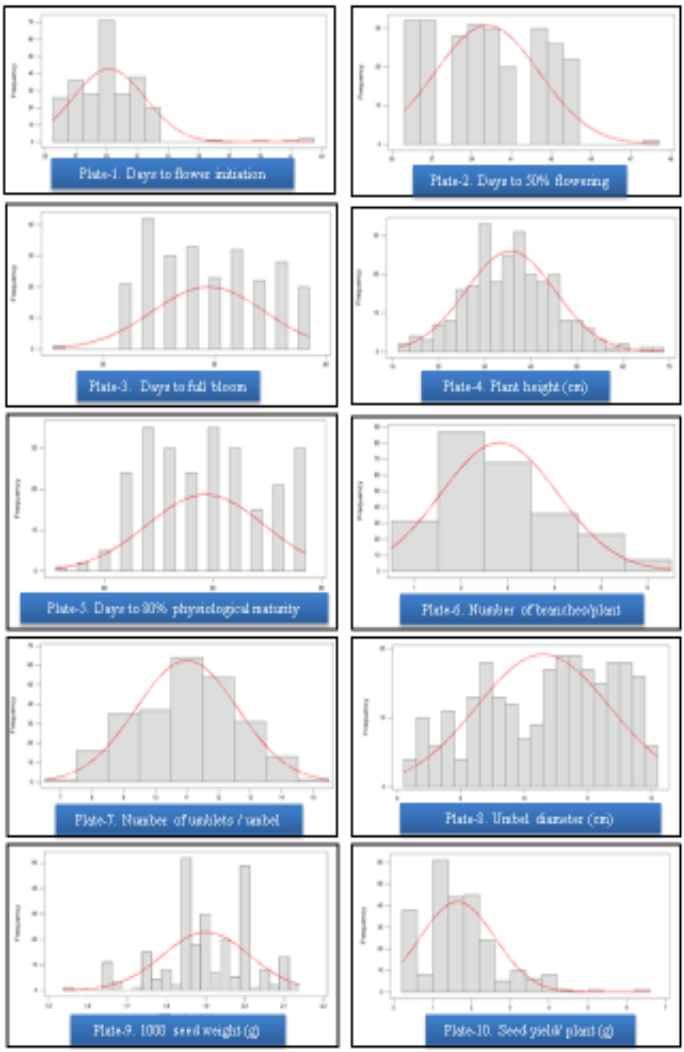


Figure 3. Expression of morphological (plate 1 to 5), yield and yield attributing traits (plate 6 to 10) in kala zeera accessions

Source: Khan *et al* (2021) Plant Genetic Resource

Diversity in flowering of Kala zeera

Diversity in pigmentation of Kala zeera

Diversity in growth habit of Kala zeera

Figure 4. Diversity in flowering, pigmentation, growth habit of kala zeera

A survey was conducted for identification of hotspots of kala zeera from high altitudes of Jammu and Kashmir and Ladakh. During the survey two hotspots were identified in district Pulwama, three in Budgam, two in Srinagar, one in Bandipora, three in Doda and three in Ladakh. A total of fifty-one (51) germplasm lines were collected from these hotspots with a sample size of twenty (20) tuber of uniform weight (>3g) for each germplasm line. In addition ninety-six (96) exotic and indigenous germplasm lines are available at Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir.

Kala zeera is harvested from the wild pockets or high hills mostly by the local people, as little efforts for domestication of this crop on scientific lines have so far been made. This existing practice of unsustainable and unscientific exploitation of this species has resulted in reduction of its natural populations and has made it a plant of special conservation concern in north-western Himalayas. Although some efforts have been done in the recent years to domesticate it in certain areas, but due to non-availability of suitable genotypes, cultivation of this crop has remained very limited. Shaung village of Kinnaur district of Himachal Pradesh is an only area where kala zeera is cultivated by the local famers with good economic returns. Here, the crop is initially propagated through seed and subsequent regenerations are possible through tubers. The cultivation

of kala zeera on scientific lines is quite remunerative as one hectare under this crop can yield net return of INR15, 000–1,00, 000 between 4^{th}–10^{th} year of its establishment. Thus, there is an urgent need for identification of suitable genotypes that can perform better under domestication or cultivation of this crop on scientific lines.

Exploration and evaluation of genetic diversity is a prerequisite for identifying the suitable genotypes in a crop, which can be utilized further for initiation of breeding programmes (Annonymous, 2004). The breeding programmes can result in development of desired elite genetic stocks for commercial cultivation. Inspite of prized herb having many therapeutic uses, the information on genetic diversity present among the different populations of *B. persicum*, especially in North-western Himalayan region is severely lacking. Similarly, information on biochemical and morphological diversity is also unavailable in this crop. The morphological characterization of many plant species is difficult because, they resemble morphologically and it is very difficult to differentiate between them (Martinez *et al*. 2007). This necessitates the use of molecular markers, particularly DNA markers for distinguishing accessions, removing duplicates, DNA fingerprinting of ambiguous genotypes, determining the extent of genetic diversity and signifying the path of domestication (Wunsch

and Hormaza 2002). However, some reports on essential oil study in Iranian populations using GC/MS technique are available but no report on populations belonging to northwestern Himalayan region exits. Therefore, it is important to determine the genetic diversity of *B. persicum* populations of North-Western Himalayan region using various morphological descriptors, randomly amplified polymorphic DNA (RAPD) markers and phytochemical parameters. The results of such study could be used to select diverse lines for cultivation of zeera out of which an elite genetic stock can further be used to develop cultivars through systematic plant breeding programmes. The standard and conformity assessment has gained utmost importance with trade globalization especially in medicinal herbs, spices and its value-added products since the non-tariff agreements such as Sanitary and Phytosanitary (SPS) and Pre-shipment Inspection (PSI) agreements insist that the product(s) is safe, free from adulterants and has the desired quality (Dhanya and Sasikumar 2010). Quality and safety assume significant importance in case of commodities which are exported out of the country because any kind of adulteration leads to rejection of consignment which in turn also affects country's prestige. Though few analytical tools are available to detect biological adulterants in medicinal herbs/ spices, they have either

low resolution power or are time consuming (Dhanya and Sasikumar 2010). To the best of our knowledge, no DNA based method has ever been reported to detect the presence of any biological adulterant in Kala zeera. DNA barcoding is a technique for rapid identification of species using small-standardized DNA fragment as a marker. Minor differences in nucleotide sequence at particular genetic loci are the key for identification in this technique. DNA barcoding reflects the distribution of inter and intra- specific variation that is separated by a distance called "DNA barcoding gap" (Hebert *et al*. 2003; Meyer and Paulay 2005). The Consortium of Barcode of Life (CBOL) coordinates DNA barcoding development and implementation universally. Apart from routine identification, DNA barcoding plays an important role in conservation biology particularly for biodiversity hotspots assessment and monitoring the international trade of rare species. Researchers are still in search of a universal barcode for plants like CO1 (cytochrome c oxidase) in animals; though several groups have successfully identified different barcode candidates for smaller taxa (Kress *et al*. 2005). The CBOL plant-working group (PWG) concluded that, plant DNA barcoding should be multi-locus, with one "anchor" (i.e. universal across the plant kingdom) and "identifiers" to distinguish closely related species. Different

scientific groups have proposed several combinations of DNA barcode regions for efficient species identification and authentication (Kress *et al*. 2005; Chase *et al*. 2007; Kress and Erickson 2007; Pennisi 2007, Zhang *et al*. 2012). Present study has been aimed at identification and standardization of DNA barcodes for unambiguous detection of bioadulteration of *kala zeera* with a low value substitute *safed zeera* (*Cumin cyminum*) wherein four different loci *viz.* ITS2, (Chen *et al.* 2010), *psbA–trnH* (Chen *et al*. 2010), *rbcL*-a (Kress *et al*. 2007) and *Cum* (Focke *et al*. 2011) representing nuclear as well as chloroplast genome were used for specific detection of *kala zeera* and its adulterant *safed zeera*.

For efficient conservation and management, the genetic composition of the species in different geographic regions needs to be assessed. RAPD markers have been used for the assessment of genetic diversity in a large number of plant species, including many red-listed plants (Li *et al*. 2002; Fu *et al*. 2003; Padmalatha and Prasad 2006b, 2007). Therefore, in the investigation of Kala zeera accessions for genetic diversity, RAPD markers could appropriately be employed.

CHEMICAL COMPOSITION AND USAGE OF KALA ZEERA

Seeds of kala zeera plant are brown/ dark brown in colour.

Bioactivity of kala zeera depends on the amount of the Thymoquinone the major component of essential oil, which is also present in fixed oil (Ali & Blunden., 2003.). Phytochemical profile of kala zeera has shown flavonoids, phenolic acids, aldehydes as well as a high content of mono-terpenes and sesqui-terpenes contained in the essential oil and extracts of this plant (Chizzola *et al*., 2014). It is used as a liver tonic, digestive, antidiarrhoeal, emmenagogue and to control parasitic infections and boost immune system (Goreja, 2003). The extracts of *B. persicum* have hypoglycemic activity and can prevent diabetes and obesity (Giancarlo *et al.* 2006). The seeds and oils of *B. persicum* are used in perfumery industries, indigenous and industrial medicine; also, they are used as spices (Panwar *et al*., 1993; Gincarlo *et al*., 2006). Seeds are stimulants and carminative and are used in treating diarrhea, dyspepsia, fever, flatulence, stomachic, hemorrhoids, hiccoughs, for antihistaminic and more numerous uses in medicine industries (Hanelt *et al*., 2001; Peter, 2004; Panda, 2004; Boskabadi and Moghaddas, 2004). This plant has several therapeutic effects on digestive and urinary tract disorders and is well known as an anti-convulsion, anthelmintic, anti-asthma and antidyspnea in Iranian folk medicine. *B. persicum* oil is capable of suppressing the initial stage of an inflammatory process (Boskabadi

and Moghaddas, 2004; Salehi *et al*., 2008). Also, this plant is used for culinary purposes and for flavoring foods and beverages (Pourmortazavi *et al*., 2005).

Kala zeera has found a significant place in the indigenous system of medicine, as the fruits are regarded as a stimulant and carminative and are useful in curing diarrhea, dyspepsia, fever, flatulence, stomache ache, haemorrhoids and obstinate hiccups (Hanelt *et al*., 2001; Peter, 2004). The seeds (fruits) have, however, been replaced by their essential oil which is now widely used for seasoning pickles, meat sauce, soups, candies, etc. Besides, being a source of essential oils rich in terpenoids and phenylpropanoids, polyene and phototoxic furanocoumarins are typical of this family. Sharififar *et al*., (2010) studied medicinal properties of *B. persicum* by evaluating the essential oil and different extracts of the seeds of this plant for antioxidant activity by Diphenylpicrylhydrazil (DPPH) assay, β-carotene bleaching and ammonium thiocyanate methods and reported its usage as antispasmodic, carminative, antiobesity and lactogage.

The oil of cumin is especially used as an antioxidant and flavor. Cuminaldehyde has been found as the characteristic constituent of the cumin seed (Lee HS, 2005) and employed advantageously in many types of flavouring preparations particularly in curries and

culinary preparations of oriental type and flavouring component such as cuminaldehyde 18.3% and perillaldehyde 8.17%, in addition to the terpenic hydrocarbons, whereas Egyptian cumin oil contains 39.2% cuminaldehyde (Srinivas, 1986). It is also used to an extent in soap perfumery and in flavouring beverages. Essential oil of *Cuminum cyminum* L. is more or less powerful external or internal antiseptic, analgesic, anti-inflammatory, haemolytic, or anti enzymatic action, sedative, stimulants and stomachics (Gruenwald, *et al*. 2004). Components may have antioxidant, anticancer, antibacterial, and larvicidal effects (Iacobellis, *et al*. 2005). Cumin may lower blood sugar, reduce seizures, strengthen bones, and treat the eye. Cumin is generally recognized as safe for human consumption as a spice and flavoring agent. The antibacterial activity of cumin Eos could attribute to high level of antimicrobial properties (Neda *et al*. 2013). The chemical composition of cumin oil from different countries includes: α-pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), P-cymene (8.5%), β-phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%) together with much smaller quantities of α-

phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral. The major components of cumin oil include: The major compounds include cumin aldehyde (p-isopropyl benzaldehyde, 25 to 35%), terpinene (29.5%), α- and β-pinene (21%), ρ-cymene (8.5%), ρ-mentha-1, 3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Perilla aldehyde, cumin alcohol, dipentene, and β-phellandrene are also present in cumin. Limonene, eugenol, α-and β- pinenes and some other minor constituents have been found in cumin oil (Gohari. R.A *et al*, 2011). The main components in the EOS (Essential oils) of green cumin are ɣ-terpinene, ɣ-terpinene-7-al and β-pinene. The chemical composition of cumin oil from different countries includes: α-pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), P-cymene (8.5%), β-phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%) together with much smaller quantities of α-phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral. Behera *et al*.,2004 studied the essential oil composition of cumin seeds after subjecting them to heating by microwaves and conventional roasting at different temperatures.

The conditions were standardized in both methods. The volatile oils distilled from these samples were analysed by GC and GC–MS. The results indicated that the microwave-heated samples showed better retention of characteristic flavor compounds, such as aldehydes, than did the conventionally roasted samples.

VALUE ADDITION IN KALA ZEERA

Black cumin also popularly known as kala or Shahi zeera in the Persian and Indian sub-continent is related variety of regular cumin and is consumed all over the world. Its seeds feature long, slender, curved, dark brown pods with distinctive earthy flavour. Kala Zeera is a basic ingredient in Kashmiri cuisine as well as very popular spice in north Indian cuisine. Black cumin especially popular for its aroma and medicinal properties, is rich in anti-inflammatory antioxidants, and is also known to be antibacterial and antiseptic. Cumin seed oil is used in perfumery and for flavouring liqueurs and cordials. Cumin seeds are also used in various ayurvedic-herbal medicines such as stomach pain, obesity, digestion and dyspepsia. It is also used in the treatment of piles, asthma, insomnia, skin disorders, respiratory disorders, bronchitis, common cold, lactation, anaemia, boils on skin and cancer. Cumin seeds have an aromatic odour and bitter taste. It is used as a condiment and is an ingredient in curry powders, seasonings of

bread, cakes and cheese. value added products like zeera powder, roasted powder, oil, drinks and food product ingredients such as zeera ladoo, zeera goli, zeera roasted powder, zeera sweet, aaloo zeera, zeera poli, zeera chicken, zeera puffs, roasted zeera bread sticks, zeera bhakhri, zeera cookie, zeera cheese pakooda, zeera biscuits and zeera cheese can be prepared from it.

Zeera Bread

Zeera Powder

Zeera Water

Zeera Rice

Zeera Chicken

Aaloo Zeera

Zeera Bhakhri

Zeera Cheese

Zeera cheese pakoda

Zeera ladoo

Zeera and value added products from zeera such as alcohol and water extract and zeera seeds as rich sources of essential oils possess many nutraceutical properties with many biological activities such as antiallergic, antioxidant, antiplatelet aggregation, hypoglycemic (Sowbhagya, 2013), antidiabetic (Lee,

2005) antihyperlipidemic (Kode *et al.*, 2005; Shirke and Jagtap, 2009), antiinflammatory (Srivastava, 1989), anticarcinogenic (Gagandeep *et al.,* 2003), immunomodulatory (Chauhan *et al.*, 2010) and relaxant properties (Boskabady *et al.*, 2005). The relaxant effect of zeera makes it a powerful bronchodilator (Prasad *et al.,* 2009), as demonstrated in guinea pig tracheal chains, with stimulation of β-adrenergic receptors, inhibition of histamine H1 receptors, or anticholinergic properties of this plant proposed as the possible mechanisms (Boskabady *et al.*, 2005).

The fruit of zeera is rich in estrogenic iso-flavonoids, luteolin, and apigenin and are effective in a variety of menopausal disorders, such as osteoporosis and cardiovascular disorders (Shirke and Jagtap, 2009). Zeera oil, due to the presence of monoterpenes like anethofuran, carvone, and limonene, inhibited benzopyrene-induced stomach tumorigenesis, 3-methylcholanthrene-induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzen- induced hepatomas in mice through

augmentation of cytochrome P450 reductase and cytochrome b5 reductase (Johri, 2011). Extracts of zeera showed hepato-protective activity in a profenofos-intoxicated group with reduced levels of liver function test (Kumar *et al.*, 2011). The volatile oils from the fruits of zeera showed antiinflammatory activity as a dose-dependent inhibition of carrageenan-induced rat paw edema (Shivakumar *et al.*, 2010). The seed extract of zeera imparted corrosion inhibition activity attributed to the presence of aromatic ring, aldehyde, and isopropyl groups and its role as a natural corrosion inhibitor on mild steel surfaces was investigated by gravimetric, potentiodynamic polarization, electrochemical impedance spectroscopy (Singh *et al.*, 2012). Sergio *et al.* (2013) demonstrated zeera essential oil (ZEO) as an active agent against *Mycobacterium tuberculosis*, with minimum inhibitory concentrations (MICs) of 6.25–12.5 μg/mL suggesting the use of the agent in ethnomedicine as an alternative remedy for symptoms of tuberculosis as well further phytochemical and pharmacological

studies for obtaining the ZEO compound effective against *M. tuberculosis*.

KALA ZEERA MARKETING IN GUREZ

Kala zeera in Kashmir, particularly in Gurez is facing marketing problems on account of absence of efficient marketing channels, lack of branding and existence of traditional post-harvest techniques. Technologies are available which can ensure the best quality of Gurez zeera, for which it is famous not only in Kashmir but in whole country and creation of spice park by Government Pampore is a step forward in this direction. The activities performed under NMHS funded research project on Kala zeera are likely to boost the overall kala zeera production of Gurez from present 129 kg^{-1} to 400 kg^{-1}. Government could frame a Kala zeera improvement policy to expand Kala zeera cultivation to non-traditional areas following technologies developed by SKUAST-K that will not only conserve the indigenous germplasm of this crop but can further enhance the overall production and

productivity of this crop. Therefore, there is a need to provide the solution to the farmers for the better market which at present is unorganized. Improving marketing value chain of kala zeera with structured marketing channels, adopt measures to stop adulteration, value addition and introduce branding of Gurez zeera will definitely increase livelihood security of more than 2000 farm families which are associated with this trade directly or indirectly. The present study is therefore an attempt to report indigenous crop diversity in kala zeera germplasm, value addition and highlight the problems of marketing of kala zeera in Kashmir valley so as to provide remedial measures and benefit the growers as much as possible and encourage their involvement in the kala zeera value chain. Kala zeera growers from Gurez could be encouraged to form Farmers' cooperatives which could be linked to a small scale industries where the value added products could be made by engaging rural youth. Such cooperatives could ultimately be liked to national and international markets which ultimately could boost

the economy of farmers and improve their livelihood security.

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NMHS Funded Research Project on Kala Zeera

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