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RESEARCH ARTICLE

Trans situ conservation of *Piper nigrum* L. in India—a review

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Abstract *Piper nigrum* L. (Piperaceae), commonly known as black pepper, is a globally cherished spice and a key player in the spice trade. This review article discusses the significance of black pepper, its diverse applications, and the need for its genetic resource conservation. Importantly, it provides the '*trans situ'* approach being followed in India for safe conservation of *P. nigrum* germplasm. This encompasses various methods of ex situ conservation, including field genebanks, seed genebanks, in vitro genebanks, and cryo genebanks. In situ conservation efforts involving on-farm practices by 'custodian farmers' are also provided. The review showcases the extensive efforts in India to conserve black pepper genetic resources through collaborative initiatives among research institutions, universities, and farming communities. Research gaps in terms of in vitro cryopreservation have been identified. Overall, this article underscores the critical importance of preserving black pepper genetic diversity to safeguard its future and support ongoing agricultural, research, and breeding endeavors.

Keywords Black pepper · Cryopreservation · Ex situ · Field genebank · In situ · In vitro

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Introduction

Piper nigrum L. (Piperaceae) (2n = 4x = 52), commonly known as black pepper, is referred to as the 'king of spices' due to its widespread use and the quantity of trade and commerce among spices in the international market (Srinivasan 2007). This robust vine thrives across diverse altitudes and exhibits remarkable adaptability to a variety of climatic gradients (Ravindran and Kallupurackal 2012). The name 'black pepper' derives from the distinctive coloration

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of its economically valued peppercorn seeds. The plant is native to India and traces its origin from the wet humid regions of the WesternGhats in southern India. Globally, the black pepper is cultivated on a total area of 678,215 hectares with the production of 793,817.98 tons (FAO 2021). India with the production of 64,815.81 tons in the cultivated area of 131,711 hectares stands out as the second leading country after Indonesia in area under cultivation and fifth in production, contributing over 8.16% of the world production (FAO 2021), with 17,958 tons earmarked for export, contributing INR 7.26 billion to the nations' foreign exchange reserves (SBI 2022). In India, Karnataka is the major producer contributing over 60.46% of the production in the country followed by Kerala (APEDA 2021).

The genus *Piper* comprises 2048 recognized species (POWO 2023), although it is *P. nigrum*, *P. longum* L. and *P. betle* L. are the three most well-known and economically important ones. Within the Indian subcontinent, a rich diversity of 114 *Piper* species exists, with 18 species nestled within the sub-mountainous Western Ghats, the contiguous peninsula, and the coastal regions (Nirmal Babu et al. 2015). The critical conservation status of *P. nigrum* becomes evident as it is listed as endangered in Andhra Pradesh (2001), near threatened in Karnataka (1999) and Tamil Nadu (1998), as revealed by the Conservation Assessment and Management Prioritization (CAMP) workshops (Gowthami et al. 2021a).

As a perennial woody climbing vine, black pepper achieves towering heights of 5–6 m through the extension of aerial roots along the support columns. Characterized by bright, lustrous leaves arranged in an alternate pattern, its most valuable part lies in the fruits (drupes), each measuring about 5 mm in diameter. These drupes constitute the prized peppercorns, the most valuable component of the plant. Black pepper is the dried fruit of a mature fruit (peppercorns) and is used in a variety of applications, mostly as a spice, condiment, preservative, pesticide, and herbal medicine (Wang et al. 2017).

The fiery flavor of black pepper, attributed to the presence of the pungent alkaloid piperine (De Almeida 2020), extends its influence beyond the culinary realm. Piperine is renowned for enhancing the bioavailability of various therapeutic drugs (Khajuria 2002) and stimulating digestive enzymes within the pancreas and intestines, notably enhancing biliary bile output (Tiwari and Singh 2008). Comprising trace quantities of safrol, pinene, sabinene, limonene, caryophyllene, and linalool, black pepper's global culinary ubiquity is unequivocal, used in a variety of cuisines worldwide.

The multifarious uses of black pepper transcend the realm of flavor, with documented roles in alleviating digestive disorders, stomach maladies, diarrhea, indigestion, respiratory ailments, intermittent fever and anxiety (Srinivasan 2007; Parganihaet al. 2011; Pany et al. 2016; Ghosh et al. 2021). Furthermore, it exhibits a protective shield against bacterial, insect, and animal infections (Scott et al. 2008; Ahmad et al. 2012). With a global reputation in ethnomedicine, black pepper is hailed for its diverse medicinal attributes, offering a spectrum of therapeutic benefits (Scott et al. 2008). Piper-amides extracts from black pepper display insecticidal properties while the secondary metabolite nerolidol assumes a pivotal role in mite control (Scott and Albert 2005). Pure compound 'Piperine' has been recognized with many more therapeutic activities such as anti-oxidant (Al-Khayri et al. 2022), anti-neoplastic/cancer (Sunila and Kuttan 2004), anti-colorectal cancer (Wu et al. 2023), anti-tumor response in breast cancer (Lasso et al. 2023), anti-pyretic (Damanhouri and Ahmad 2014), anti-apoptotic (Pathak and Khandelwal 2007), anti-hypertensive (Saleem et al. 2022), anti-platelets (Taqvi et al. 2008), anti-spasmodic (Tiwari et al. 2023), anti-metastatic (Manoharan et al. 2009), anti-mutagenic (EI-Hamas et al. 2003; Zahin et al. 2021), neuroprotective effect (Pany et al. 2016), anti-spermatogenic (Chinta and Periyasamy 2016), anti-depressant (Lee et al. 2005; Ghosh et al. 2021), anti-asthmatics (Parganiha et al. 2011), anti-thyroid (Panda and Kar 2003), free-radical scavenger (Gülçin 2005), hepatoprotective (Mushtaq et al. 2021), immune-stimulator (Pathak and Khandelwal 2009), antibacterial (Dorman and Deans 2000), anti-fungal (Zhang et al. 2021), insecticidal and larvicidal activities (Park 2012; Saleem et al. 2022). Apart from this piperine also minimizes the likelihood of arthrosclerosis through anti-atherogenic and hypolipidemic effects (Yang et al. 2019). It is purportedly used to treat gastrointestinal disorders, lung problems, fever, cold and colic disorders (Parmar et al. 1997; Ravindran 2000).

Due to the overutilization of *P. nigrum*, its germplasm conservation is paramount to safeguard genetic diversity and guaranteeing its continuous availability for future generations. In this review, an attempt has been made to capture the trans situ (both in situ and ex situ) methods of conservation of black pepper for future agricultural, research, or breeding purposes (Agrawal et al. 2023).

Information on propagation and conservation were drawn through the literature available on the current status of the black pepper crop in India. It included information on black pepper cultivar and varietal diversity in the states of Karnataka and Kerala. Furthermore, a survey to document on-farm conservation of black pepper in the central Western Ghats of Karnataka, India was also conducted during November 2020 to November 2021 (Fig. 1). Data on on-farm conservation of *P. nigrum* was documented from the different custodian farmers using a questionnaire and semi-structured interview. The data was collected through the snowball sampling technique where, custodian farmers were selected using information shared by other fellow farmers (Subedi et al. 2003). A global positioning system (GPS) was used to define and record the coordinates (latitude, longitude) of each survey area following on-farm conservation of blackpepper landraces (Rathi et al. 2019). In addition, the custodian farmers were interviewed in order to record their personal data (name, age, occupation, address, and contact information), farm details (cropping pattern, total farm area, landrace cultivation area), barriers to cultivation, community knowledge of on-farm germplasm conservation and biodiversity conservation, current and historical landrace use, and traditional uses associated with the landraces.

Plant propagation

Black pepper is a climber, typically propagated through various methods, including seed germination, cuttings (2–6 nodes per cutting), layering, and grafting (Ravindran 2000). However, seed propagation poses considerable challenges. The seeds exhibit a short viability window of approximately one week

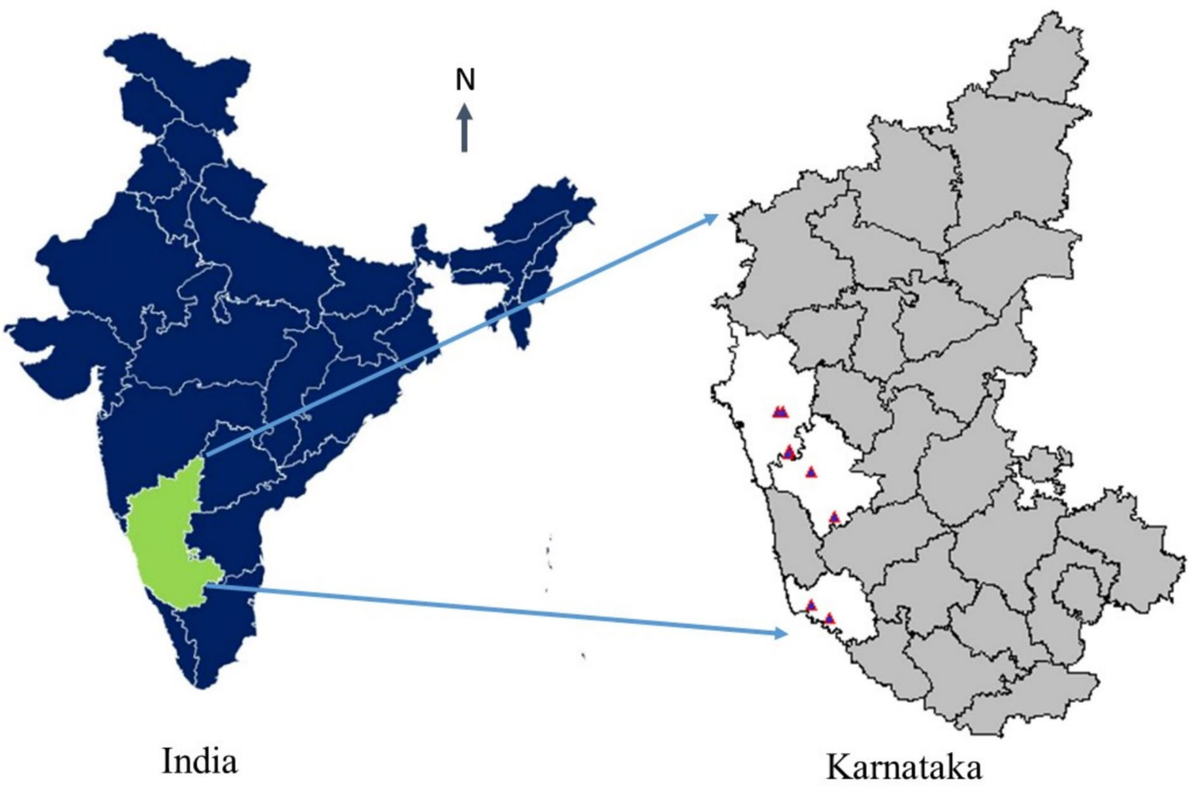

Fig. 1 Map depicting the survey areas to document on-farm conservation of *P. nigrum* in Karnataka, India (Co-ordinates for the farmers are provided in Table 3)

after harvest and are particularly susceptible to deterioration when exposed to moisture-depleted storage conditions (Atal and Banga 1962; Ravindran et al. 2000). Furthermore, seed propagation yields a small number of genetically diverse progenies due to low seed viability and high sterility in the post-fertilization phases (Hussain et al. 2011), and also seed propagation takes a long time and is troublesome (Atal and Banga 1962). Thus, traditionally, black pepper propagation predominantly relies on cuttings with 2–6 nodes, layering, and grafting for field cultivation (Abbasi et al. 2010). Cuttings are favored for their efficiency, while other propagation approaches are less prevalent due to their slow and time-intensive nature (Hussain et al. 2011; Nair and Gupta 2003).

Black pepper is commonly prone to various pathogens, such as fungi, bacteria, viruses, and mycoplasma, which pose significant challenges to conventional propagation techniques (Ravindran and Kallupurackal 2012). Internal viral and mycoplasma infections are particularly troublesome as they can be transmitted through vegetative reproduction methods (Philip et al. 1992). Consequently, the limitations of conventional propagation methods hinder the ability to meet the growing demand for planting material.

Despite its substantial economic importance, India's black pepper productivity lags behind that of other pepper-producing countries. This disparity can be related to the insufficient availability of quality planting material from high-yielding cultivars. Additionally, diseases such as foot rot and anthracnose, and insects such as the pollu beetle (*Longitarsus nigripennis*) further impede vegetative cultivation (Ravindran and Kallupurackal 2012). Two viruses, namely, cucumber mosaic virus and piper yellow mosaic virus are of concern as they can be transmitted to offspring plants, exacerbating the challenges faced in black pepper cultivation (Bhat et al. 2018).

Genetic diversity

The intraspecific diversity in *P. nigrum* might be due to the occurrence of the species in a wide range of altitudinal zones and its high adaptability to a broad range of environmental conditions. In terms of breeding behavior, black pepper predominantly relies on self-pollination (geitonogamy) due to its floral structure, sequential bloom patterns, and long pollen viability, despite some protogyny and potential for outcrossing (Krishnamoorthy and Parthasarathy 2009). While wind and insects might play a minor role, selfing with occasional inter-flower fertilization within the vine reigns supreme for this spice plant. Vegetative/clonal propagation is the most common practice for successful propagation and survival of various lines. The primary gene pool of black pepper is made up of landraces, natural mutations, improved cultivars, and even true seedlings (Joy et al. 2007; Sasikumar et al. 2007). The current cultivar diversity in *P. nigrum* has been evolved by accidental selection from natural hybridization and highly successful vegetative propagation, clonal selection from landraces based on economically important traits, and development of hybrids, which resultedin a great deal of varietal distinction in fruit size, shape, and fruiting behavior (Krishnamoorthy and Parthasarathy 2010).

An assessment of the genetic diversity of germplasm is essential for both its efficient utilization and conservation. Several researchers have conducted in-depth analyses to determine the genetic diversity of the Indian germplasm of *P. nigrum* utilizing morphological, biochemical, and molecular markers. The investigations that have been conducted based on morphological and biochemical parameters have demonstrated that black pepper genepool has diverse intraspecific variants found in both wild and cultivated populations (Mathai et al. 1981; Chandy et al. 1984; Raju et al. 1983; Kanakaswamy et al. 1985; Ravindran et al. 1997; Ravindran and Kallupurackal 2001; Kurian et al. 2002; Mathew et al. 2006; Parthasarathy et al. 2006; Zachariah and Parthasarathy 2008; Sruthi et al. 2013; Preethy et al. 2018).

Several investigations have been conducted to assess the extent of genetic variation among Indian black pepper cultivars using molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplifiable Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) (Pradeepkumar et al. 2003; George et al. 2005; Joy et al. 2007, 2011; Raghavan et al. 2010; Sen et al. 2010; Sheeja et al. 2013; Jagtap et al. 2016; Jose et al. 2017).

Considerable diversity among *P. nigrum* genotypes, cultivars, landraces, advanced cultivars and wild accessions has been reported (Joy et al. 2007, 2011; Raghavan et al. 2010; Sheeja et al. 2013). Using RAPD markers, *P. columbrinum* has been proven to be distantly related to *P. nigrum* and *P. longum*. In addition, *P. nigrum* landraces grown in southern and northern parts of coastal India could be distinctly segregated (Pradeepkumar et al. 2003). Distinct fingerprint profiles have been identified in the Karimunda cultivar (Joy et al. 2007) and SSR based specific bands have been identified in different black pepper accessions (Raghavan et al. 2010). Differences at the species level between two parents of *Piper* species including the varietal difference among various genotypes of black pepper have been identified using SSR markers (Jagtap et al. 2016).

Kumari et al. (2019) performed the first whole-genome SSR mining in black pepper and provided the results in the form of a database called PinigSSRdb (http://www.nbpgr.ernet.in:9091/index.php). From the assembled genomic sequence, a total of 69,126 SSRs were found, with one SSR for every 6.3 kb due to the SSR frequency of 158 per MB. Dinucleotides were the most common form of microsatellite repeat motif, accounting for 48.6% of the total, followed by trinucleotides (23.7%) and compound repeats (20.62%). For validation, a set of 85 SSRs was employed, and 74 of these yielded amplification products with the anticipated size. However, these putative SSR marker sites are anonymous loci (unknown chromosomal position) as they are based on 916 scaffolds rather than 26 chromosomes. Later, using ddRAD (double digest Restriction Associated DNA) based genotyping-by-sequencing (GBS) for rapid discovery of genome-wide location-specific polymorphic SSR markers (Negi et al. 2022), the first web-genomic resources, BlackP2MSATdb, was developed. This contains all 276,230 putative SSR markers found throughout the whole genome of black pepper, with an average distance of 2.76 kilobases between SSRs and a relative density of 362.88 SSRs per Mb. The black pepper reference genome shared 3176 polymorphic markers with 29 genotypes in total, of which 2015 were hypervariable. Subsequent sequencing of the chloroplast genome (Gaikwad et al. 2023) revealed that, the size of the chloroplast genome was 161,522 bp, showing a quadripartite structure consisting of 89,153 bp large single copy (LSC) area and 18,255 bp small single copy (SSC) region, which are divided by 27,057 bp long copy of inverted repeats (IRs). Furthermore, 216 SSRs were found, and 11 of these were confirmed by amplification in 12 different *Piper* species. These molecular investigations have laid the foundation for the effective utilization of black pepper germplasm, identification of quantitative trait loci, map-based cloning, molecular breeding through marker assisted selection, evolutionary research and further insights to the omics.

Even though there are more than 100 known cultivars, many of them are facing the risk of extinction for various reasons. These factors include the decimation of pepper crops by diseases such as foot rot and gradual decline, as well as replacement of the traditional cultivars by high yielding cultivars. The highest concentration of cultivars is found in the state of Kerala, followed by Karnataka, as indicated in Tables 1 and 2. Most of these cultivars are bisexual forms. The Western Ghats region is very

Table 1 Major black pepper cultivars in Kerala and Karnataka. (*Source* Mathew et al. 2006; Prasath et al. 2011; Pannaga et al. 2021; Reshma et al. 2022)

State	Cultivar diversity
Kerala	*Ampirian*, *Angamali*, *Arakkulammunda*, *Aranavalan*, *Arieepadappan*, *Arivally*, *Attamuriyan*, *Arayanmunda*, *Arimulak*, *Balankotta*, *Chengannurkodi*, *Cheppakulamundi*, *Cheriyakaniakadan*, *Cheriyauthiran*,*Cheriyikoda*, *Cherumany*, *Cholamundi*, *Chumala*, *Irumaniyan*, *Jeerakamundi*, *Kalluvally*, *Kallubalankotta*, *Kallumany*, *Kalyanamandiram*, *Kaniakkadan*, *Kangiramkodan*, *Karieelanchi*, *Karimunda*, *karimkotta*, *Karivally*, *Kottan*, *Kottanadan*, *Kumbhakodi*, *Kuthiravally*, *Kuttianikodi*, *Kuruvantherivally*, *Malamundi*, *Manjamundi*, *Marampadarthi*, *Murithothan*, *Muttiyaramundi*, *Mundi*, *Nadan*, *Nateshankodi*, *Narayakodi*, *Neelamundi*, *Nedumchola*, *Neyyattinkaramundi*, *Orumaniyan*, *Pala*, *Panickaruvally*, *Padarppan*, *Perambramunda*, *Peringamala*, *Perumkodi*, *Poojaranmunda*, *Thevanmundi*, *Thippalikodi*, *Thommankodi*, *Thirimuriyan*, *Thulamundi*, *Uthiran*, *Uthirankotta*, *Vadakkan*, *Valliyakaniyakadan*, *Vattamundi*, *Vellanamban*, *Valiyaranmunda*, *Wayanadan*
Karnataka	*Ademane*, *Bilimallegesara*, *Daddayya*, *Huchmenasu*, *Huklakai special*, *Haavli special*, *Keregaddemalligesara*, *Kurimalai*, *Kuriyalamundi*, *Karimaratta*, *Karimalligesara*, *Madana*, *Malligesara*, *Sigandhini*, *Uddagara*, *Vokkalu*, *Workalamorata*

Table 2 Released cultivars of black pepper in India. (*Source* Aravind et al. 2022; https://kau.in/basic-page/varieties-released)

S.No	Variety	Pedigree/parentage	Institute	Year of Release
1	*Panniyur* 1*	*Uthirankotta* × *Cheriyakaniyakadan*	Pepper Research Station, Kerala Agricultural University, Panniyur	1971
2	*Panniyur* 2#	Open pollinated progeny of *Balankotta*		1990
3	*Panniyur* 3	*Uthirankotta* × *Cheriyakaniyakadan*		1990
4	*Panniyur* 4#	*Kuthiravally* type II		1990
5	*Panniyur* 5#	Open pollinated progeny of *Perumkodi*		1996
6	*Panniyur* 6#	*Karimunda* type III		2001
7	*Panniyur* 7	Open pollinated progeny of *Kalluvally*		2001
8	*Panniyur* 8*	*Panniyur* 6 × *Panniyur* 5		2013
9	*Panniyur*9	Open pollinated progeny selectionof*Panniyur 3*		2018
10	*Panniyur*10	*Panniyur 1* × *Cul 54*(Open pollinated of progeny of*Kalluvally*)		2019
11	*Vijay*	*Panniyur 2* × *Neelamundi*	College of Horticulture, Vellanikkara, Kerala	2013
12	PLD-2#	*Kottanadan*	Regional Centre, Indian Institute of Oil Palm Research, Palode	1996
13	*Sreekara*#	*Karimunda* (KS 14)	IISR, Calicut	1990
14	*Subhakara*#	*Karimunda*		1990
15	*Panchami*#	*Aimpiriyan*		1991
16	*Pournami*#	*Ottaplackal* 1		1991
17	IISR *Thevam*#	*Thevamundi*		2004
18	IISR *Malabar Excel*	*Cholamundi* × *Panniyur* 1		2004
19	IISR *Girimunda*	*Narayakodii* × *Neelamundi*		2004
20	IISR *Shakthi*	Open pollinated progeny of *Perambramundi*		2004
21	*Arka Coorg Excel*	Seedling selection	ICAR-Indian Institute of Horticulture Research, CHES, Chettali and ICAR-IISR, Regional Station, Appangala, Karnataka	2012

#Clonal selection from *Inter-cultivar hybrid

high in endemic species (Nirmal Babu et al. 2015). Unfortunately, it is also among the most ecologically threatened area due to large-scale encroachments and human settlements that have proliferated over the past century. Sen et al. (2016) conducted ecological niche modeling, which revealed a shift in the niche centroid's direction and a decline in the area of suitable habitats for black pepper in the southern Western Ghats under both projected climate change scenarios for 2080. According to this scenario's analysis, trans situ conservation of *P. nigrum* germplasm is crucial for safe conservation of these highly valuable resources.

Trans situ conservation of *P. nigrum*: Indian efforts

Trans situ conservation represents a holistic methodology that combines diverse in situ and ex situ approaches (Dempewolf et al. 2014; Gowthami et al. 2021b; Agrawal et al. 2023). It integrates three important components: (1) in situ protection, management, and research; (2) local and regional seed and living plant collections for conservation, research, and education; and (3) national and global genebanks for plant breeding and crop research. While many nations employ complementary conservation strategies for various species, the predominant approach typically leans towards ex situ measures. In most instances, in situ and ex situ conservation efforts remain disjointed and independent. Trans situ conservation approaches transcend these limitations, facilitating the successful integration of multiple in situ and ex situ conservation, research, and education activities at the local, national, and global levels. Trans situ conservation of black pepper genetic resources in India has been accomplished via the collaboration of ICAR institutes, State Agricultural Universities, Central Universities and farming communities (Fig. 2).

In situ conservation

In situ conservation involves conservation of plant genetic resources (PGR) within their natural habitats, allowing these conserved species to co-evolve with the environmental changes (Maxted et al. 2001). This crucial strategy safeguards genetic resources through various means, such as biosphere reserves, forest reserves, botanical gardens, national parks, and on-farm conservation.On-farm conservation involves farmers cultivating and managing a wide range of populations, such as locally produced traditional crop cultivars, as well as associated wild and weedy species or forms, in agro-ecosystems where a crop has evolved. This approach serves to protect a wide range of traditional types that are well-suited for low-input farming and offer resilience in the face of climate change adaptation (Hodgkin and Hamilton1993). More than 50 traditional cultivars are being conserved in the on-farm by the farmers of Kerala (Mathew et al. 2006; Reshma et al. 2022). Community Agrobiodiversity Centre, Wayanad has been recognized by the Kerala State Council for Science, Technology, and Environment (KSCSTE) as a grant-in-aid institution for enhancing research and extension activities for the conservation and sustainable utilization of cultivated and wild species of pepper, including other native crops (MSSRF 2021).

To explore the practice of on-farm conservation in the context of black pepper, we conducted a survey in

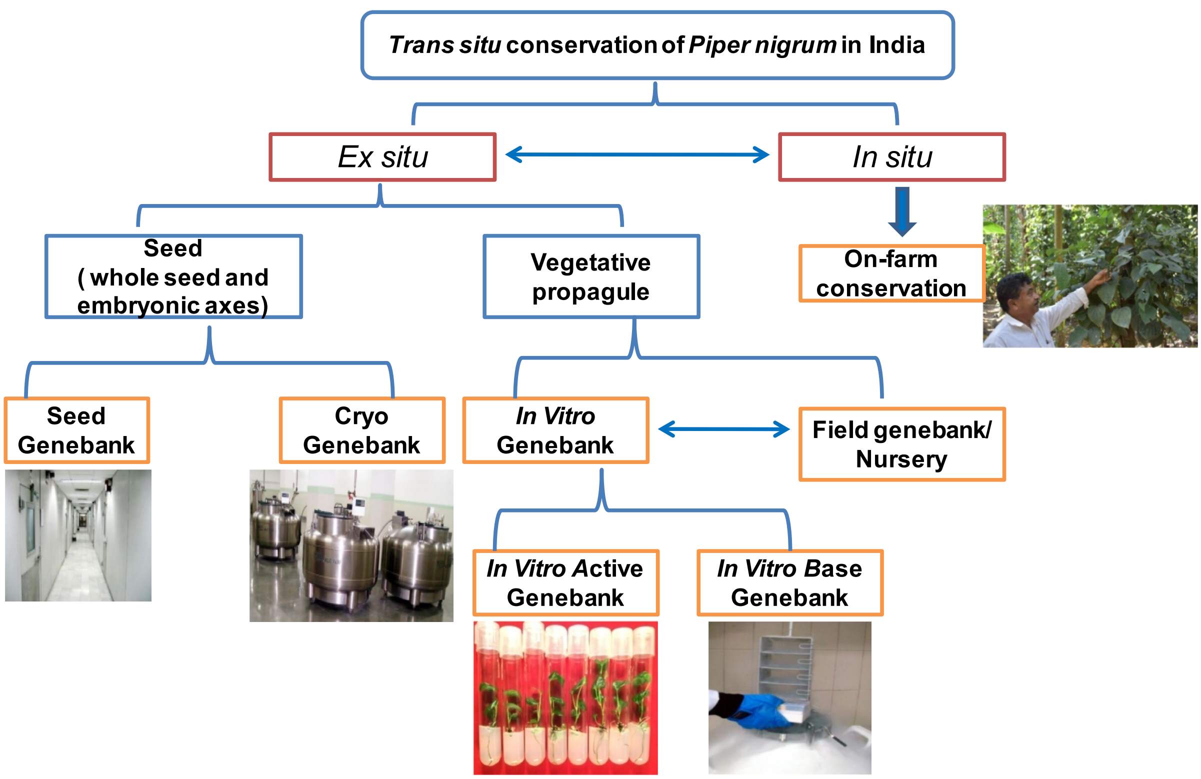

Fig. 2 Trans situ conservation of black pepper genetic resources in India

the central Western Ghats region of Karnataka, covering three districts viz., Uttara Kannada, Shivamogga, Dakshina Kannada. During the survey, we identified nine dedicated 'custodian farmers' who have been actively engaged in on-farm conservation of different cultivars/landraces spanning multiple generations. These custodian farmers are instrumental in preserving various black pepper cultivars and landraces, including but not limited to *Ademane*, *Baalehalli*, *Bilemallige Sara*, *BiliMunda*, *Doddoge*, *Gejje Hipli*, *Karimallige Sara*, *Karimunda*, *Karimenasu*, *Kudurugunta*, *Kurimale*, *Maliyaali Gere*, *Malabar*, *Mallige sara*, *Motakare*, *Neelamundi*, *Okkalu*, *Sigandini*, *Thekkam BunchPepper*, *Thiruchugere*, *Uddagere*, *Uddakarki* and *Vakkalu* (see Table 3 and Fig. 3 for details). Farmers revealed that they are conserving

Table 3 List of custodian farmers practicing on-farm conservation of black pepper landraces/ cultivars

S.No	Name of custodian farmer	Place of on-farm conservation	Geographic location		Landraces/cultivars conserved
			Latitude	Longitude	
1	Ramakrishna Gajanan Bhat	Uttara Kannada	14.67105	74.76405	*Ademane*, *Baalehalli*, *BilemalligeSara*, *Doddoge*, *KarimalligeSara*, *Karimunda*, *Kurimale*, *Motakare*, *Okkalu*, *Uddakarki*
2	Mangala Murthy MS	Uttara Kannada	14.65805	74.71903	*Baalehalli*, *Kudurugunta*, *Kurimale*, *Neelamundi*, *Sigandini*, *Thiruchugere*, *Uddagere*
3	Vittal Ramakrishna Sett	Uttara Kannada	14.29698	74.84156	*BiliMunda*, *Karimenasu*, *Karimunda*
4	Subramanya MN	Shivamogga	13.66441	75.26666	*GejjeHipli*
5	AP Sadashiva	Dakshina Kannada	12.71183	75.21177	*Karimunda*, *Maliyaali Gere*
6	Vasanth Kaje	Dakshina Kannada	12.82979	75.03582	*Karimunda*
7	Vaasudeva K Naik	Uttara Kannada	14.27171	74.82141	*Malabar*
8	Ramesh Hegde	Uttara Kannada	14.66007	74.76604	*Mallige sara*, *Vakkalu*
9	Natesh	Shivamogga	14.09475	75.03698	*Thekkam Bunch Pepper*

Fig. 3 On-farm conservation of black pepper (*P. nigrum)* germplasm is being practiced by custodian farmers (**A**–**D**); Ramesh Hegde, Uttara Kannada (Sirsi), Karnataka, India from last two generations (**A**) AP Sadashiva, Dakshina Kannada (Puttur), Karnataka, India from 2–3 generations (**B**) Natesh, Shivamogga (Sagara), Karnataka from one generation (**C**) Vaasudeva K Naik, Uttara Kannada (Siddapur), Karnataka, India from 2–3 generations (**D**)

these landraces for several years over the generations for different objectives including conservation, yield parameters, quality of the pepper in terms of pungency and tolerance to pests and diseases. Additionally, farmers are also involved in exchange of these landraces with fellow farmers of the region.

Ex situ conservation

Ex situ conservation refers to the preservation of plant and animal species outside of their natural habitat (Panis et al. 2020). Common approaches include field genebanks (whole plants in the field, primarily for clonally propagated, recalcitrant seeded species, and forest crops), seed genebanks (orthodox seeds crops at low temperatures), in vitro genebanks (vegetatively propagated plants, species that do not produce seeds, produce recalcitrant seeds, and plants with long juvenile periods in vitro under slow-growth conditions), cryogenebanks (non-orthodox seeded species, vegetatively propagated).

Seed genebank

Plant species have been conserved by nature through inherent seeds during millions of years of evolution. Seed storage in seed genebanks at low temperature is the most convenient and often employed approach ex situ conservation strategy of seed producing crops (Hay and Sershen 2021). However, black pepper seeds are classified as 'recalcitrant' as seed viability decreases with reduction in moisture content below ~ 12% (Chaudhury and Chandel 1994). Hence, storage of pepper germplasm in seedbanks is not practical as they are vegetatively propagated and seeds are recalcitrant and heterozygous.

Field genebank

A field genebank is an ex situ conservation approach in which species are collected and transported to a secondary location away from their original location where they can be planted conserved as living collections in semi-isolated conditions, where natural evolution and adaptation processes are either temporarily halted or altered by introducing the specimen to an unnatural habitat with suppressed selection pressures in order for it to survive and be conserved (Panis et al. 2020). Field genebanks often have significantly more individuals per accession than botanic gardens. The primary application is for the conservation and utilization of species that exhibit the following characteristics. (a) do not produce bankable seeds (non-orthodox seeded species); (b) have extensive life cycles, making bringing up material for regular research from a seed collection problematic; (c) are generally propagated vegetatively/clonally; (d) produce few seeds, and (e) threatened and exceptional plant species (Agrawal et al. 2023). Exceptional plant species includes, species with insufficient available viable seed to preserve a minimum sustainable population of a species, species with seeds that are intolerant to desiccation at 15% RH, species with seeds that are partially desiccation tolerant but have a P50 (the time at which 50% of the seeds have died) of < 20 years at − 18/− 20 °C, and species with seeds with very long germination times (> 1 year) or those for which germination has not yet been successful with any conventional dormancy-breaking methods (Pence et al. 2022).

Systematic efforts have been made since 1976 at the ICAR-Indian Institute of Spices Research (IISR), Calicut, Kerala, to collect indigenous germplasm of black pepper and its wild relatives. The collections span regions of Western Ghats forests from Maharashtra to Kerala including Goa, Karnataka and Tamil Nadu, the Andaman and Nicobar Islands, and the North Eastern regions of India, in addition to the key pepper growing tracts of South India (ICAR-IISR 2009). The collected germplasm has been characterized for morphological, yield, biotic, abiotic, and quality characteristics (ICAR-IISR 2022).

The ICAR-IISR established a National Repository for the *exsitu* conservation of black pepper germplasm (Ravindran and Nirmal Babu 1994). Because of the threat of disease and pests (for example, Phytophthora foot rot and nematodes), conservation approach at the ICAR-IISR has been four-pronged:

Nursery Genebank: Each accession is trailed in serial order and is continuously multiplied using the serpentine approach.
Clonal repository: This repository maintains 10 rooted cuttings of each accession.

Field Genebank: Accessions are planted for preliminary yield assessment, characterization, and evaluation.

In vitro Genebank: Accessions are conserved using tissue culture methods.

The ICAR-IISR Experimental Farm in Peruvannamuzhi, Kerala, houses the black pepper germplasm field genebank, and alternate field genebanks are maintained at Central Horticultural Experiment Station (CHES), Chettalli, Karnataka, and Regional Station of IISR at Chelavoor, Kerala. These repositories collectively host approximately 3466 black pepper accessions(ICAR-IISR 2022). This makes ICAR-IISR the largest repository of black pepper germplasm in the world, which includes over 1375 hybrids and 120 open-pollinated progeny (OP) lines.

Additionally, black pepper germplasm accessions are also being maintained at All India Coordinated Research Project on Spices (AICRPS) at the Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kerala; Pepper Research Station, Sirsi, Karnataka; University of Agricultural Research, Dharwad, Karnataka; Regional Research Station, Chintapally, Acharya N G Ranga Agricultural University (ANGRAU), Andhra Pradesh; and Horticultural Research Station, Yercaud, Tamil Nadu. To provide additional safeguard to the germplasm, two alternate centers have been identified at ICAR-Central Plantation Crops Research Institute (CPCRI), Seed farm at Kidu, Karnataka and Cardamom Research Centre CRC of IISR at Appangala, Karnataka for cultivar and wild germplasm, respectively. Duplicate set of germplasm will be established in these centers in a phased manner. The information about maintenance of germplasm various AICRPS centres is presented in Table 4. ICAR-NBPGR, Regional Station, Thrissur, Kerala also maintains 98 accessions of *P. nigrum* in the field genebank.

In vitro conservation

Due to the heterozygous nature of its seeds, black pepper is primarily propagated by stem cuttings. However, this method is slow, time-consuming, and susceptible to biotic and abiotic stresses in the field. To address these challenges and facilitate the multiplication and conservation of black pepper germplasm, in vitro clonal propagation protocols have been developed (Nirmal Babu et al. 1999; Tyagi et al. 1998, 2004). To achieve in vitro conservation in in vitro genebank, several researchers standardized micropropagation protocols either by direct or indirect organogenesis viz., callus and somatic embryos (Nair and Gupta 2003), shoot tips (Nazeem et al. 1992; Philip et al. 1992; Joseph et al. 1996; Nirmal Babu et al. 1997, 2007), nodal explants (Bhat et al. 1995), and leaf explants (Sujatha et al. 2003). Recently, Deepak et al. (2022) developed an effective micropropagation protocol by culturing nodal explants in MS media enriched with 0.5 mg L^{-1} BAP with a satisfactory

Table 4 Black pepper germplasm maintained at various AICRPS centres. (*Source* AICRPS 2021)

Centre	Indigenous collections		Exotic	Total
	Cultivated	Wild & related species		
Kerala Agricultural University, Ambalavayal	30	–	–	30
Regional Agricultural Research Station (Acharya N.G. Ranga Agricultural University) Chintapalle	26	–	–	26
Department of Horticulture (Konkan Krishi Vidya Peeth), Dapoli	60	–	–	60
Pepper Research Station (Kerala Agricultural University) Panniyur	343	57	3	403
Department of Horticulture (Uttar Banga Krishi Viswa Vidyalaya, North Bengal Campus) Pundibari	22	–	–	22
Agricultural Research Station (Pepper), Sirsi	258	7	1	266
Horticultural Research Station, Yercaud	34	3	–	37
Kerala Agricultural University, Pampadumpara	52	–	–	52
Total	825	67	4	896

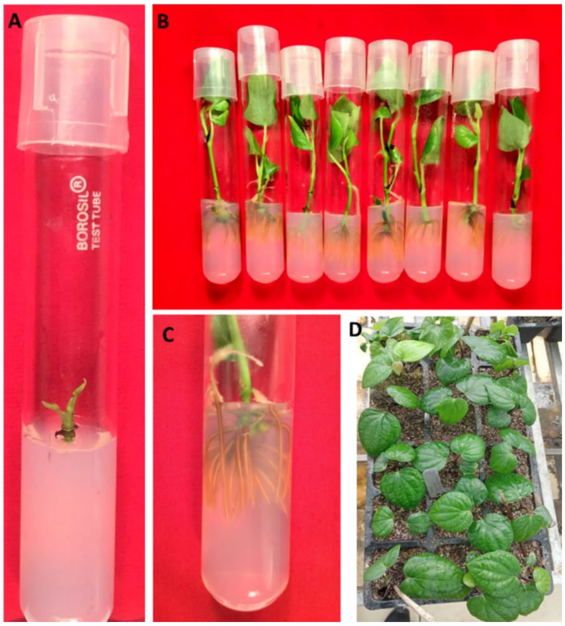

Fig. 4 In vitro conservation of *Piper nigrum* L. in In Vitro Genebank of ICAR-NBPGR, New Delhi (**A**–**C**); Stages of in vitro establishment (**A**) (15 days after inoculation), multiplication (**B**) (3 months after inoculation) and rooting (**C**) (4 months after inoculation), (**D**) Ex vitro hardening of in vitro cultures (3 week after transplanting)

rate of multiplication (15,600 plants from 12 nodal segments per year) that are easily acclimatized to the field conditions (Fig. 4).

The primary goal of in vitro conservation is to reduce frequent demand for subculture, which can be accomplished in two ways: (i) maintaining cultures under 'normal growth' in standard culture room conditions (SCC) comprising 25 ± 2 °C temperature, 16-h light/8-h dark photoperiod and light intensity of 40 µmol m^{-2} s^{-1}, and (ii) maintaining cultures under growth-limiting strategies (slow growth strategies) (Agrawal et al. 2023).At ICAR-IISR, Calicut, black pepper shoot tip cultures could be stored up to 360 days without subculture in half strength Woody Plant Medium (WPM) with 15 g/L each of sucrose and mannitol in screw capped culture tubes with 85% survival (Nirmal Babu et al. 1999). In the In Vitro Genebank at ICAR-NBPGR, New Delhi, 1,985 accessions of around 150 species belonging to six crop groups including (i) tropical fruits (449 accessions), (ii) temperate and minor tropical fruits (390 accessions), (iii) tuber crops (527 accessions), (iv) bulbous crops (178 accessions), (v) medicinal & aromatic plants (211 accessions) and (vi) spices and industrial crops (230 accessions), which include seven accessions of *Piper nigrum* are being conserved in vitro. In vitro cultures are being conservedon minimal media(½ MS + 0.1 mg/l IAA) for 11 months (Tyagi et al. 1998; Deepak 2022).

Cryo Genebank

In vitro conservation within in vitro gene bank faces several challenges, including the need for frequent subculturing, limitations in terms of duration, and the susceptibility of explants to endogenous bacterial contamination during in vitro clonal multiplication (Philip et al. 1992; Bhat et al. 1995; Abbasi et al. 2010; Rani and Dantu 2016). To address these limitations and ensure safe and long-term conservation, a complementary strategy is required. Cryopreservation stands out as the sole technology available for the secure, long-term conservation of plant genetic resources (Sharma et al. 2019; Panis et al. 2020; Agrawal et al. 2022a, b).

So far, cryopreservation of *P. nigrum* has been reported using seeds and seed-derived somatic embryos (Chaudhury and Chandel 1994; Decruse and Seeni 2003; Chaudhury and Malik 2004; Yamuna 2007; Nirmal Babu et al. 2012) (Table 5). Cryo Genebank of ICAR-NBPGR, New Delhi, is a unique multi-crop genebank, where protocol was standardized for cryopreservation of *P. nigrum* seeds by desiccating to 12% and 6% moisture contents, storing them in liquid nitrogen (− 196 °C), achieving survival rates of 45% and 10.5%, respectively (Chaudhury and Chandel 1994). Thereafter, the protocol was applied to cryopreserve 102 accessions of *P. nigrum* using seeds, except one accession which was cryopreserved using both seeds and embryonic axes (Table 6).

Yamuna (2007) reported somatic embryos cryopreservation by the application of encapsulation-dehydration and vitrification techniques. In encapsulation-dehydration treatment, optimal post-thaw survival rate of 62% was achieved by preculturing in 0.7 M sucrose for one day, further dehydration in the laminar air flow for 6 h to attain 21% moisture content. In the vitrification procedure, the somatic embryos were precultured for 3 days on Schenk & Hildebrandt (SH) basal salt medium with 0.3 M

Table 5 Cryopreservation research in black pepper

Explant	Technique/ parameters of cryopreservation	Survival/ regrowth	Reference
P. nigrum seeds	Desiccated to 12% and 6% moisture content (MC)	Survival rates of 45% and 10.5%	Chaudhury and Chandel (1994)
P. nigrum seeds	Desiccated to 12.8 ± 0.48% (wild), 13.3 ± 0.16% MC (*Karimunda* cv, *Panniyoor*-1 cv, *Kottanadan* cv)	Germination of 85.0 ± 15.0% (wild), 60.0 ± 14.9% (*Karimunda* cv), 73.3 ± 12.5% (*Panniyoor*-1 cv), 68.8 ± 19.0% (*Kottanadan* cv)	Decruse and Seeni (2003)
Encapsulated seed derived somatic embryos	Encapsulation-dehydration	62% regrowth	Yamuna (2007)
Encapsulated seed derived somatic embryos	Vitrification	71% regrowth	Yamuna (2007)

Table 6 Cryopreserved accessions of black pepper from Kerala, Karnataka and Tamil Nadu conserved at cry genebank at ICAR-NBPGR, New Delhi. (*Source* http://www.nbpgr.ernet.in:8080/cryobank/Datasearch.aspx)

Source of material (no. of accessions)	Accessions cryopreserved
Kerala	
Idukki (26)	IC85320, IC85334, IC85335, IC85338, IC85354, IC85357, IC85363, IC85376, IC85387, IC85353, IC85351, IC85331, IC85381, IC85361, IC85339, IC85336, IC85318, IC85386, IC85321, IC85384, IC85313, IC85370, IC85375, IC85324, IC85341, IC360239
Kannur (21)	IC266446, IC266442, IC266445, IC266456, IC266455, IC406460, IC266447, IC266451, IC266452, IC266459, IC266460*, IC85528, IC85533, IC85536, IC85537, IC85538, IC85545, IC85546, IC406461, IC85544, IC85530
Kollam (5)	IC373755, IC266417, IC373831, IC373833, IC373832, IC373828, IC266414, IC266415, IC266416, IC373837, IC373830, IC373827, IC373770, IC373754
Kottayam (5)	IC85427, IC85433, IC85434, IC85435, IC85443
Kozhikode (1)	IC266462
Malappuram (2)	IC266401, IC266399
Palakkad (14)	IC266397, IC266394, IC360236, IC266402, IC266403
Pathanamthitta (15)	IC266409, IC85396, IC85397, IC85398, IC85401, IC85402, IC85406, IC85410, IC85418, IC85422, IC85428, IC85415, IC85403, IC85429, IC85392
Thiruvananthapuram (1)	IC373748
Karnataka	
Dakshina Kannada (3)	IC266411, IC266438, IC266437
Kodagu (6)	IC266418, IC266379, IC266381, IC266382, IC266383, IC85571
Uttara Kannada (1)	IC85526
–	IC360244
Tamil Nadu	
Kanyakumari (1)	IC373783

sucrose and subjected to vitrification treatment for 60 min at 25 °C, resulting in a 71% survival rate after cryopreservation. RAPD and ISSR profiling were used to demonstrate the genetic fidelity of the conserved somatic embryos (Table 5).

However, it is important to note that in black pepper selfing with occasional outcrossing is the predominant mode of pollination (Sasikumar et al. 1992), and seed conservation only helps to conserve gene pools. To conserve genotypes in clonal crops like black pepper, it is essential to conserve clonal explants such as shoot tips and buds to maintain clonal identity and regenerate plants with higher genetic stability compared to cell suspensions, embryogenic tissue, and callus (Wang et al. 2021). Consequently, experiments were conducted at ICAR-NBPGR to standardize a cryopreservation protocol using shoot tips and nodal explants with the aim of maintaining the true-to-type characteristics of the material (Deepak 2022). However, the results were suboptimal, indicating the need for further experimentation.

Conclusion

Conservation of black pepper (*Piper nigrum*) germplasm is important to maintain genetic diversity and ensure its availability for future generations. Its unique flavor, culinary versatility, and diverse medicinal properties make it a cherished commodity. However, the overexploitation of *P. nigrum* has raised concerns about its conservation and genetic diversity. To address the challenges of conserving valuable genetic resources of *P. nigrum*, extensive efforts have been made in India through trans situ conservation, by integrating all realms of both ex situ and in situ conservation methods. The establishment of field genebanks, seed genebanks, in vitro gene banks, and cryo genebanks have played a pivotal role in safeguarding black pepper germplasm. Additionally, on-farm conservation practices involving custodian farmers have contributed significantly to maintaining diverse black pepper cultivars and landraces. Research over the last three decades has yielded rich dividends, and > 5,000 accessions, cultivars are being maintained in India at various labs, research stations and farmers' fields. More research is warranted to safely duplicate this material using in vitro and cryopreservation techniques, especially for elite cultivars and unique lines that require clonal maintenance. The collaborative efforts of research institutions, universities, and farming communities in India exemplify a holistic approach to safeguarding this valuable genetic resource.

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Author contribution AA and SA: Conceptualization and supervision, manuscript editing. DAD and GMP: Experimental work, data analysis and writing original draft, prepared Fig. 2 and 3. RG & MS: Investigation, writing review and editing; prepared Fig. 1. SC & EVM: Review & editing. All authors have reviewed and approved the manuscript.

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Data availability All data generated during this study are included in the paper.

Declarations

Conflict of interest The authors declare no competing interests.

References

Abbasi HB, Nisar A, Hina F, Tariq M (2010) Conventional and modern propagation techniques in *Piper nigrum*. J Med Plants Res 4(1):7–12. https://doi.org/10.5897/JMPR09.025

Agrawal A, Gowthami R, Chander S, Srivastava V (2022a) Sustainability of in vitro genebanks and cryo gene banks. Indian J Plant Genet Resour 35:180–184. https://doi.org/10.5958/0976-1926.2022.00065.1

Agrawal A, Sharma N, Gupta S, Bansal S, Srivastava V, Malhotra EV, Chander S, Gowthami R, Singh K (2022b) Biotechnological applications for plant germplasm conservation at ICAR-National Bureau of Plant Genetic Resources, India—recent achievements. Acta Hort. https://doi.org/10.17660/ActaHortic.2022.1339.5

Agrawal A, Gowthami R, Srivastava V, Chander S (2023) Trans-situ conservation of PGR. In: Gautam RK et al (eds) Plant Genetic Resources Management-Theory and Practice. ICAR-National Bureau of Plant Genetic Resources, New Delhi, pp 48–52

Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA (2012) Biological role of *Piper nigrum* L. (Black pepper): a review. Asian Pacific J Trop Biomed 2(3):S1945–S1953. https://doi.org/10.1016/S2221-1691(12)60524-3

AICRPS (2021) Annual Report 2021. Aravind S et al (eds). ICAR-All India Coordinated Research Project on Spices, ICAR-IISR, Kozhikode, Kerala, India

Al-Khayri JM, Upadhya V, Pai SR, Naik PM, Al-Mssallem MQ, Alessa FM (2022) Comparative quantification of the phenolic compounds, piperine content, and total polyphenols along with the antioxidant activities in the *Piper trichostachyon* and *P. nigrum*. Molecules 27(18):5965. https://doi.org/10.3390/molecules27185965

APEDA (2021) https://agriexchange.apeda.gov.in/India%20Production/India_Productions.aspx?hscode=1096. Accessed on 21.10.2023

Aravind S, Prasath D, Sankar MS, Shivakumar MS, Akshitha HJ, Aarthi S, Saji KV, Rema J (2022) Spices Varieties of ICAR-IISR. ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India

Atal CK, Banga SS (1962) Phytochemical studies on stem of *P. longum*. Indian J Pharm 24:105

Bhat SR, Chandel KPS, Malik SK (1995) Plant regeneration from various explant of cultivated *Piper* species. Plant Cell Rep 14(6):398–402. https://doi.org/10.1007/BF00238605

Bhat AI, Biju CN, Srinivasan V, Ankegowda SJ, Krishnamurthy KS (2018) Current status of viral diseases affecting black pepper and cardamom. J Spices Aromatic Crops 27(1):1–16. https://doi.org/10.25081/josac.2018.v27.i1.1009

Chandy KC, Neelakantan Potty N, Kanna K (1984) Parameters for varietal classification of pepper. Indian Spices 21:18–22

Chaudhury R, Chandel KPS (1994) Germination studies and cryopreservation of seeds of black pepper (*Piper nigrum* L.): a recalcitrant species. CryoLetters 15:145–150

Chaudhury R, Malik SK (2004) Genetic conservation of plantation crops and spices using cryopreservation. Indian J Biotech 3:348–358

Chinta G, Periyasamy L (2016) Reversible anti-spermatogenic effect of piperine on epididymis and seminal vesicles of albino rats. Drug Res 66(08):420–426. https://doi.org/10.1055/s-0042-108186

Damanhouri ZA, Ahmad A (2014) A review on therapeutic potential of *Piper nigrum* L. (Black Pepper): the King of Spices. Med Aromat Plants 3(3):161. https://doi.org/10.4172/2167-0412.1000161

De Almeida GC, Oliveira LF, Predes D, Fokoue HH, Kuster RM, Oliveira FL, Abreu JG (2020) Piperine suppresses the Wnt/β-catenin pathway and has anti-cancer effects on colorectal cancer cells. Sci Rep 10(1):1–12. https://doi.org/10.1038/s41598-020-68574-2

Decruse SW, Seeni S (2003) Seed cryopreservation is a suitable storage procedure for a range of *Piper* species. Seed Sci Technol 31:213–217. https://doi.org/10.15258/sst.2003.31.1.23

Deepak DA, Malhotra EV, Shankar M, Agrawal A (2022) Improved micropropagation protocol and molecular marker based genetic stability assessment of black pepper (*Piper nigrum* L.). Indian J Plant Genet Resour 35(2):264–274. https://doi.org/10.5958/0976-1926.2022.00030.4

Deepak DA (2022) *In vitro* clonal propagation and cryopreservation protocol development in *Piper* species for germplasm conservation. PhD Thesis, Division of Plant Genetic Resources, Post-Graduate School, Indian Agricultural Research Institute (Deemed University), New Delhi, India

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecol Sustain Food Sys 38(4):369–377. https://doi.org/10.1080/21683565.2013.870629

Dorman HD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J App Microbiol 88(2):308–316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

El-Hamas R, Idaomar M, Alonso-Moraga A, Serrano AM (2003) Antimutagenic properties of bell and black peppers. Food Chem Toxic 41(1):41–47. https://doi.org/10.1016/S0278-6915(02)00216-8

FAO (2021) FAOSTAT, https://www.fao.org/faostat/en/#data/QCL. Accessed on 21.10.2023

Gaikwad AB, Kaila T, Maurya A, Kumari R, Rangan P, Wankhede DP, Bhat KV (2023) The chloroplast genome of black pepper (*Piper nigrum* L.) and its comparative analysis with related piper species. Front Plant Sci 13:1095781. https://doi.org/10.3389/fpls.2022.1095781

George KJ, Ganga G, Varma RS, Sasikumar B, Saji KV (2005) Identification of hybrids in black pepper (*Piper nigrum* L.) using male parent-specific RAPD markers. Curr Sci 88(2):216–218

Ghosh S, Kumar A, Sachan N, Chandra P (2021) Anxiolytic and antidepressant-like effects of essential oil from the fruits of *Piper nigrum* Linn. (Black pepper) in mice: involvement of serotonergic but not GABAergic transmission system. Heliyon 7(4):e06884. https://doi.org/10.1016/j.heliyon.2021.e06884

Gowthami R, Sharma N, Pandey R, Agrawal A (2021a) Status and consolidated list of threatened medicinal plants of India. Genet Resour Crop Evol 68:2235–2263. https://doi.org/10.1007/s10722-021-01199-0

Gowthami R, Sharma N, Pandey R, Agrawal A (2021b) A model for integrated approach to germplasm conservation of Asian lotus (*Nelumbo nucifera* Gaertn.). Genet Resour Crop Evol 68:1269–1282. https://doi.org/10.1007/s10722-021-01111-w

Gülçin İ (2005) The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. Int J Food Sci Nutr 56(7):491–499. https://doi.org/10.1080/09637480500450248

Hay FR, Sershen N (2021) New technologies to improve the ex situ conservation of plant genetic resources. Burleigh Dodds Science Publishing Limited, Cambridge, pp 1–32. https://doi.org/10.19103/AS.2020.0085.14

Hodgkin EP, Hamilton BH (1993) Fertilizers and eutrophication in southwestern Australia: setting the scene. Fert Res 36:95–103. https://doi.org/10.1007/BF00747579

Hussain A, Naz S, Nazir H, Shinwari ZK (2011) Tissue culture of black pepper (*Piper nigrum* L.) in Pakistan. Pak J Bot 43(2):1069–1078

ICAR-IISR (2009) http://spices.res.in/mail/rpf2009/Gen.%20I.%20813-Collrction-%20black%20pepper.pdf. Accessed on 07.07.2023

ICAR-IISR (2022) Annual Report of Indian Institute of Spice Research. Praveen R et al. (eds), ICAR-Indian Institute of Spice Research, Kozhikode, Kerala, India

Jagtap AB, Sujatha R, Nazeem PA, Meena OP, Pathania S (2016) Morpho-molecular characterization of putative interspecific crosses in black pepper (*Piper nigrum* L. and *Piper colubrinum*). Plant Omics 9(1):73–80

Jose S, Sujatha R, Deeshma KP (2017) Novel EST-SSR marker development and validation in black pepper cultivars and varieties. J of Trop Agric 55(2):175–179

Joseph L, Nazeem PA, Thampi MS, Philip S, Balachandran M (1996) In vitro techniques for mass multiplication of black pepper (*Piper nigrum* L.) and ex vitro performance of the plantlets. J Plant Crops 24:511–516

Joy N, Abraham Z, Soniya EV (2007) A preliminary assessment of genetic relationships among agronomically important cultivars of black pepper. BMC Genet 8(1):1–7. https://doi.org/10.1186/1471-2156-8-42

Joy N, Prasanth VP, Soniya EV (2011) Microsatellite based analysis of genetic diversity of popular black pepper genotypes in South India. Genetica 139:1033–1043. https://doi.org/10.1007/s10709-011-9605-x

Kanakaswamy MT, Narayanan Namboodiri KM, Luckins CB (1985) Key for identification of different cultivars of pepper. Indian Cocoa, Arecanut Spices J 9:6–11

Khajuria A, Thusu N, Zutshi U (2002) Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultrastructure and enzyme kinetics. Phytomedicine 9(3):224–231. https://doi.org/10.1078/0944-7113-00114

Krishnamoorthy B, Parthasarathy VA (2009) Improvement of black pepper. CAB Rev. 4, 85. https://cabidigitallibrary.org/doi/https://doi.org/10.1079/PAVSNNR20105003

Krishnamoorthy B, Parthasarathy VA (2010) Improvement of black pepper. CABI Reviews 28(2010):1–2. https://doi.org/10.1079/PAVSNNR20105003

Kumari R, Wankhede DP, Bajpai A, Maurya A, Prasad K, Gautam D, Rangan P, Latha M, John KJ, Bhat KV, Gaikwad AB (2019) Genome wide identification and characterization of microsatellite markers in black pepper (*Piper nigrum*): a valuable resource for boosting genomics applications. PLoS ONE 14(12):e0226002. https://doi.org/10.1371/journal.pone.0226002

Kurian PS, Backiyarani S, Josephrajkumar A, Murugan M (2002) Varietal evaluation of black pepper (*Piper nigrum* L.) for yield, quality and anthracnose disease resistance in Idukki District Kerala. J Spices Aromat Crops 11(2):122–124

Lasso P, Rojas L, Arévalo C, Urueña C, Murillo N, Nossa P, Sandoval T, Chitiva LC, Barreto A, Costa GM, Fiorentino S (2023) *Piper nigrum* extract suppresses tumor growth and enhances the antitumor immune response in murine models of breast cancer and melanoma. Cancer Immunol Immunother 72(10):3279–3292. https://doi.org/10.1007/s00262-023-03487-3

Lee SA, Hong SS, Han XH, Hwang JS, Oh GJ, Lee KS, Ro JS (2005) Piperine from the fruits of *Piper longum* with inhibitory effect on monoamine oxidase and antidepressant-like activity. Chem Pharma Bull 53(7):832–835. https://doi.org/10.1248/cpb.53.832

Manoharan S, Balakrishnan S, Menon VP, Alias LM, Reena AR (2009) Chemopreventive efficacy of curcumin and piperine during 7, 12-dimethylbenz (a) anthracene-induced hamster buccal pouch carcinogenesis. Singap Med J 50(2):139

Mathai CK, Kumaran PM, Chandy KC (1981) Evaluation of commercially important chemical constituents in wild black pepper types. Plant Foods Hum Nutr 30(3–4):199–202. https://doi.org/10.1007/BF01094024

Mathew PJ, Mathew PM, Kumar V (2006) Multivariate analysis in fifty cultivars/landraces of 'black pepper' (*Piper nigrum* L.) occurring in Kerala, India. In: proceedings of international symposium on breeding research on medicinal and aromatic plants pp 180–185

Maxted N, Tan A, Amri A, Valkoun J (2001) In situ conservation. In: Maxted N, Bennett SJ (eds) Plant Genetic Resources of Legumes in the Mediterranean. Current Plant Science and Biotechnology in Agriculture, Springer, Dordrecht

MSSRF (2021) Thirty-First Annual Report 2020–2021. M. S. Swaminathan Research Foundation Centre for Research on Sustainable Agricultural and Rural Development Chennai, India, pp 37

Mushtaq A, Aslam B, Muhammad F, Khan JA (2021) Hepatoprotective activity of *Nigella sativa* and *Piper nigrum* against Concanavalin A-induced acute liver injury in mouse model. Pak Vet J 41(1):78–84. https://doi.org/10.29261/pakvetj/2020.076

Nair RR, Gupta SD (2003) Somatic embryogenesis and plant regeneration in black pepper (*Piper nigrum* L.): Direct somatic embryogenesis from tissues of germinating seeds and ontogeny of somatic embryos. J Hortic Sci Biotechnol 78(3):416–421. https://doi.org/10.1080/14620316.2003.11511641

Nazeem PA, Joseph L, Geetha CK, Sreekandannair G (1992) In vitro techniques for cloning of black pepper, *Piper nigrum* L. J Plant Crops 20:257–257

Neelam Sharma R, Gowthami RP (2019) Synthetic seeds: a valuable adjunct for conservation of medicinal plants. In: Faisal M, Alatar AA (eds) Synthetic Seeds: Germplasm Regeneration, Preservation and Prospects. Springer International Publishing, Cham, pp 181–216. https://doi.org/10.1007/978-3-030-24631-0_7

Negi A, Singh K, Jaiswal S, Kokkat JG, Angadi UB, Iquebal MA, Umadevi P, Rai A, Kumar D (2022) Rapid genome-wide location-specific polymorphic SSR marker discovery in black pepper by GBS approach. Front Plant Sci 13:846937. https://doi.org/10.3389/fpls.2022.846937

Nirmal Babu K, Geetha SP, Minoo D, Ravindran PN, Peter KV (1999) In vitro conservation of germplasm. In: Ghosh SP (ed) Biotechnology and its Application in Horticulture. Narosa Publishing House, New Delhi, pp 106–129

Nirmal Babu K, Geetha SP, Minoo D, Yamuna G, Praveen K, Ravindran PN, Peter KV (2007) Conservation of spices genetic resources through in vitro conservation and cryopreservation. In: Peter KV, Abraham Z (eds) Biodiversity in Horticultural Crops, vol 1. Daya Publishing House, New Delhi, pp 210–233

Nirmal Babu K, Yamuna G, Praveen K, Minoo D, Ravindran PN, Peter KV (2012) Cryopreservation of spices genetic resources. In: Katkov I (ed) Current Frontiers in Cryobiology. Intech, Rijeka, pp 457–484. https://doi.org/10.5772/35401

Nirmal Babu K, Sastry EVD, Saji KV, Minoo Divakaran HJ, Akshitha S, Aarthi A, Sharon PN, Ravindran KV, Peter, (2015) Diversity and erosion in genetic resources of spices. In: Ahuja MR, Mohan Jain S (eds) Genetic diversity and erosion in plants. Springer International Publishing, Cham, pp 225–261. https://doi.org/10.1007/978-3-319-25637-5_9

Nirmal Babu K, Ravindran PN, Peter KV (1997) Protocols for Micropropagation of Spices and Aromatic Crops. Indian Institute of Spices Research, Calicut, India.

Panda S, Kar A (2003) Piperine lowers the serum concentrations of thyroid hormones, glucose and hepatic 5′ D activity in adult male mice. Hormone Metabolic Res 35(9):523–526. https://doi.org/10.1055/s-2003-42652

Panis B, Nagel M, Van den Houwe I (2020) Challenges and prospects for the conservation of crop genetic resources in field genebanks, in in vitro collections and/or in liquid nitrogen. Plants 9(12):1634. https://doi.org/10.3390/plants9121634

Pannaga TS, Narayanpur VB, Hiremath JS, Hegde L, Gandolkar K, Rathod V, Chandrakala R (2021) Evaluation of black pepper (*Piper nigrum* L.) cultivars for yield and quality parameters under hill zone of Karnataka. J Pharmacogn Phytochem 10(1):1497–1500

Pany S, Pal A, Sahu P (2016) Potential neuroprotective effect of piperine in pilocarpine-induced temporal lobe epilepsy. Indo Am J Pharm Res 6:4369–4375

Parganiha R, Verma S, Chandrakar S, Pal S, Sawarkar HA, Kashyap P (2011) In vitro anti-asthmatic activity of fruit extract of *Piper nigrum* (Piperaceae). Int J Herbal Drug Res 1:15–18

Park IK (2012) Insecticidal activity of isobutyl amides derived from *Piper nigrum* against adult of two mosquito species, *Culex pipiens* pallens and *Aedes aegypti*. Nat Prod Res 26(22):2129–2131. https://doi.org/10.1080/14786419.2011.628178

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Boll PM (1997) Phytochemistry of the genus Piper. Phytochemistry 46(4):597–673. https://doi.org/10.1016/S0031-9422(97)00328-2

Parthasarathy U, Saji KV, Jayarajan K, Parthasarathy VA (2006) Biodiversity of Piper in South India–application of GIS and cluster analysis. Curr Sci 91(5):652–658

Pathak N, Khandelwal S (2007) Cytoprotective and immunomodulating properties of piperine on murine splenocytes: an in vitro study. European J Pharma 576(1–3):160–170. https://doi.org/10.1016/j.ejphar.2007.07.033

Pathak N, Khandelwal S (2009) Immunomodulatory role of piperine in cadmium induced thymic atrophy and splenomegaly in mice. Environ Toxic Pharma 28(1):52–60. https://doi.org/10.1016/j.etap.2009.02.003

Pence VC, Meyer A, Linsky J, Gratzfeld J, Pritchard HW, Westwood M, Bruns EB (2022) Defining exceptional species—A conceptual framework to expand and advance ex situ conservation of plant diversity beyond conventional seed banking. Biol Conserv 266:109440. https://doi.org/10.1016/j.biocon.2021.109440

Philip VJ, Joseph D, Triggs GS, Dickinson NM (1992) Micropropagation of black pepper (*Piper nigrum* Linn.) through shoot tip cultures. Plant Cell Rep 12(1):41–44. https://doi.org/10.1007/BF00232421

POWO (2023) https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:326982-2#source-KB. Accessed on 18.10.2023

Pradeepkumar T, Karihaloo JL, Archak S, Baldev A (2003) Analysis of genetic diversity in *Piper nigrum* L. using RAPD markers. Genet Resour Crop Evol 50:469–475. https://doi.org/10.1023/A:1023917809042

Prasath D, Saji KV, Sasikumar B, Krishnamoorthy B, Anandaraj M (2011) Genetic diversity and conservation in major spices. In Krishnamurthy et al (eds) In: Souvenir and Abstracts, National Sympsoium on Spices and Aromatic Crops (SYMSAC VI): Exploiting Spices Production Potential of the Deccan Region. Indian Society for Spices, Kozhikode, Kerala, India, pp 41–58

Preethy TT, Aswathy TS, Sathyan T, Dhanya MK, Murugan M (1970) Performance, diversity analysis and character association of black pepper (*Piper nigrum* L.) accessions in the high altitude of Idukki district, Kerala. Journal of Spices and Aromatic Crops 27:17. https://doi.org/10.25081/josac.2018.v27.i1.1010

Raghavan R, Elumalai S, Babu KN, Hittalmani S (2010) Molecular characterization of black pepper (*Piper nigrum*) using RAPD and SSR markers. Biosci Biotechnol Res Asia 7(2):1011–1015

Raju K, Ravindran PN, Nair MK (1983) Quality evaluation of black pepper cultivars. Indian Spices 20(1):3–5

Rani D, Dantu PK (2016) Sustained shoot multiplication and method for overcoming in vitro browning in medicinally important plant, *Piper chaba* Hunt. Proc Natl Acad Sci-India, Sect B: Biol Sci 86(2):407–413. https://doi.org/10.1007/s40011-014-0461-1

Rathi RS, Yadav SK, Bhatt KC, Panwar NS, Semwal DP, Ahlawat SP (2019) On-farm Conservation of rainfed rice landrace diversity in Chhattisgarh India. Indian J Plant Genet Resour 32(1):1–10. https://doi.org/10.5958/0976-1926.2019.00001.9

Ravindran PN (ed) (2000) Black Pepper: *Piper nigrum*. CRC Press, Boca Raton

Ravindran PN, Kallupurackal JA (2001) Black pepper. In: Peter KV (ed) Handbook of Herbs and Spices. Woodhead Publishing Limited Abington Hall, Cambridge, pp 62–95

Ravindran PN, Kallupurackal JA (2012) Black pepper. In: Peter KV (ed) Handbook of Herbs and Spices. Woodhead Publishing, Cambridge, pp 86–115

Ravindran PN, Nirmal Babu K (1994) Genetic resources of black pepper. In: Chadha KL, Rethinum P (eds) Advances in Horticulture, vol 9. Malhotra Publishing House, New Delhi, pp 99–120

Ravindran PN, Balakrishnan R, Nirmal Babu K (1997) Morphometrical studies on black pepper (*Piper nigrum* L.) cluster analysis of black pepper cultivars. J Spices Arom Crops 6(1):9–20

Reshma P, Neethu RS, Sreekala GS (2022) Genetic diversity of black pepper (*Piper nigrum* L.) in India: a review. J Pharm Innov 11(10):832–839

Saleem A, Naureen I, Naeem M, Tasleem G, Ahmed H, Farooq U (2022) Therapeutic role of *Piper nigrum* L. (black pepper) and pharmacological activities. Sch Int J Biochem 5(1):15–21. https://doi.org/10.36348/sijb.2022.v05i01.003

Sasikumar B, George JK, Ravindran PN (1992) Breeding behaviour of black pepper. Indian J Genet Plant Breed 52(01):17–21

Sasikumar B, George JK, Saji KV, Gowda ASJ, Zachariah T (2007) Two unique black pepper accessions with very long spikes from the centre of origin. Plant Genet Res: Charact Util 13(2):183–185. https://doi.org/10.1017/S147926211400080X

SBI (2022) Spice Board India. https://indianspices.com/sites/default/files/major%20itemwise%20export%202023%20web.pdf. Accessed on 21.10.2023

Scott IM, Jensen HR, Philogène BJ, Arnason JT (2008) A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. Phytochem Rev 7(1):65–75. https://doi.org/10.1007/s11101-006-9058-5

Scott D, Albert LL (2005) Maize metallothionein promoter. Patent Publication n. WO/2005/063997.

Sen S, Skaria R, Muneer PA (2010) Genetic diversity analysis in *Piper* species (Piperaceae) using RAPD markers. Mol Biotechnol 46(1):72–79. https://doi.org/10.1007/s12033-010-9281-6

Sen S, Gode A, Ramanujam S, Ravikanth G, Aravind NA (2016) Modeling the impact of climate change on wild *Piper nigrum* (Black Pepper) in Western Ghats, India using ecological niche models. J Plant Res 129:1033–1040. https://doi.org/10.1007/s10265-016-0859-3

Sheeja TE, Uma G, Sasikumar B, Saji KV, Rahul PR (2013) Genetic diversity study in *Piper* spp. using inter simple sequence repeat (ISSR) markers. J Spice Aromat Crops 22(2):111–119

Srinivasan K (2007) Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Critical Rev Food Sci Nutri 47(8):735–748. https://doi.org/10.1080/10408390601062054

Sruthi D, Zachariah JT, Leela NK, Jayarajan K (2013) Correlation between chemical profiles of black pepper (*Piper nigrum* L.) var. Panniyur-1 collected from different locations. J Med Plant Res 7(31):2349–2357. https://doi.org/10.5897/JMPR2013.4493

Subedi A, Chaudhary P, Baniya BK, Rana RB, Tiwari RK, Rijal DK, Sthapit BR, Jarvis DI (2003) Who maintains crop genetic diversity and how? Implications for on-farm conservation and utilization. Cult Agric 25(2):41–50

Sujatha R, Babu LC, Nazeem PA (2003) Histology of organogenesis from callus cultures of black pepper (*Piper nigrum* L.). J Trop Agric 41:16–19

Sunila ES, Kuttan G (2004) Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. J Ethnopharma 90(2–3):339–346. https://doi.org/10.1016/j.jep.2003.10.016

Taqvi SI, Shah AJ, Gilani AH (2008) Blood pressure lowering and vasomodulator effects of piperine. J Cardiovasc Pharmacol 52:452–458. https://doi.org/10.1097/FJC.0b013e31818d07c0

Tiwari P, Singh D (2008) Antitrichomonas activity of sapindussaponins, a candidate for development as microbicidal contraceptive. J Antimicrobial Chemother 62:526–534. https://doi.org/10.1093/jac/dkn223

Tiwari BR, Inamdar MN, Orfali R, Alshehri A, Alghamdi A, Almadani ME, Alshehri S, Rabbani SI, Asdaq SMB (2023) Comparative evaluation of the potential antispasmodic activity of *Piper longum*, *P. nigrum*, *Terminalia bellerica*, *T. chebula* and *Zingiber officinale* in experimental animals. Saudi Pharma J 31(9):101705. https://doi.org/10.1016/j.jsps.2023.101705

Tyagi RK, Bhat SR, Chandel KPS (1998) *In vitro* conservation strategies for spices crop germplasm *Zingiber, Curcuma* and *Piper* species. In: Mathew NM, Kuruvila JC (eds) Developments in Plantation Crop Research. Rubber Research Institute of India, Kerala, pp 77–82

Tyagi RK, Abraham Z, Latha M, Velayudhan KC, Ravindran PN, George JK, Agrawal A, Dhillon BS (2004) Conservation of spices germplasm in India. Indian J Plant Genet Resour 17:163–174

Wang Y, Li R, Jiang ZT, Tan J, Tang SH, Li TT, Zhang XC (2017) Green and solvent-free simultaneous ultrasonic-microwave assisted extraction of essential oil from white and black peppers. Industrial Crops Prod 114:164–172. https://doi.org/10.1016/j.indcrop.2018.02.002

Wang MR, Bi W, Shukla MR, Ren L, Hamborg Z, Blystad DR, Wang QC (2021) Epigenetic and genetic integrity, metabolic stability, and field performance of cryopreserved plants. Plants 10(9):1889. https://doi.org/10.3390/plants10091889

Wu R, Zhao J, Wei P, Tang M, Ma Z, Zhao Y, Li Du, Wan L (2023) *Piper nigrum* extract inhibits the growth of human colorectal cancer HT-29 cells by inducing p53-mediated apoptosis. Pharmaceuticals 16(9):1325. https://doi.org/10.3390/ph16091325

Yamuna G (2007) Studies on Cryopreservation of Spices Genetic Resources. PhD Thesis, University of Calicut, Kerala, India.

Yang Y, Kanev D, Nedeva R, Jozwik A, Rollinger JM, Grzybek W, Atanasov AG (2019) Black pepper dietary supplementation increases high-density lipoprotein (HDL) levels in pigs. Curr Res Biotechnol 1:28–33. https://doi.org/10.1016/j.crbiot.2019.08.002

Zachariah TJ, Parthasarathy VA (2008) Black pepper. In: Parthasarathy VA, Chempakam B, Zachariah TJ (eds) Chemistry of Spices. CABI, UK, pp 21–40. https://doi.org/10.1079/9781845934057.0021

Zahin M, Najat AB, Iqbal A, Fohad MH, Abdullah SA, Mashael WA, Kahkashan P, Misfera S (2021) Antioxidant, antibacterial, and antimutagenic activity of *Piper nigrum* seeds extracts. Saudi J Biol Sci 28(9):5094–5105. https://doi.org/10.1016/j.sjbs.2021.05.030

Zhang C, Zhao J, Famous E, Pan S, Peng X, Tian J (2021) Antioxidant, hepatoprotective and antifungal activities of black pepper (*Piper nigrum* L.) essential oil. Food Chem 346:128845. https://doi.org/10.1016/j.foodchem.2020.128845