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Review

Improvement of black pepper

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Abstract

Black pepper (*Piper nigrum* L., Piperaceae) – 'King of spices' – is one of the oldest spices known. It originated in the humid, tropical evergreen forests of Western Ghats of India and is now grown in more than 25 countries, particularly in tropics. Important growing locations are India, Indonesia, Malaysia, Brazil, Thailand, Sri Lanka, Vietnam and China. There are several production constraints faced by farmers seeking to achieve sustainable yield. Global climate change, inadequate water availability, labour scarcity, coupled with biotic stresses such as epidemic diseases and pests, are the important challenges to be addressed by scientists. In addition to these, consumers expect clean and high-quality spices. In this context, the goal of improvement of black pepper should be for bold berries with high quality and research should also be oriented to producing crop with lower levels of pesticide residues, contamination with adulterants and mycotoxins. The genetic resources of this crop in India are a great strength. This germplasm, containing local cultivars, wild forms collected from the area of origin and related species are a wealth to be utilized for crop improvement. Cultivar diversity is one of the principal components of diversity in black pepper and over 100 black pepper cultivars are established in India. The main breeding objectives are high yield, with resistance to biotic and abiotic stresses, coupled with good quality parameters. The paper deals with breeding characteristics of black pepper and effective breeding strategy. Black pepper is a vine, predominantly self-pollinated and commercially cultivated through orthotropic stem cuttings. Conventionally, clonal selection is the main breeding method and historically it was adopted by the British for introducing the crop into their colonial countries, and this practice continued for a long time. Each producing country has its own selections. Research efforts have been focused on hybridization and polyploid breeding and micropropagation. Panniyur 1 is a famous hybrid and it is cultivated in many production centres. Now molecular approaches are used to develop input-responsive varieties with biotic and abiotic resistance for higher yield and quality.

AQ2 **Keywords:** ?

Introduction

Black pepper (*Piper nigrum*) is perhaps the oldest spice known to the world. Black pepper originated in the humid, tropical evergreen forests of Western Ghats of South India, was domesticated thousands of years ago and is now grown in many tropical countries apart from India, such as Indonesia, Malaysia, Brazil, Sri Lanka, Vietnam, China, etc. The efforts of European countries helped to spread this crop in their colonial countries wherever a favourable climate for growth and production existed. Currently it is grown in 26 countries, with a production of 369 587 MT from 467 708 ha having a productivity of 790.2 kg/ha [1]. A steady increase in area (R^2=0.8958) and production (R^2=0.8714) was noted from 1961 to 2004. However, the change in productivity is lower (R^2=0.3699) for the same period. The area, production and productivity vary among countries. Area varies from less than 100 ha to more than 2 000 000 ha, production also varies between less than 100 MT to more than

Table 1 Black pepper production from major producing countries (tonnes)

Country	Brazil	India	Indonesia	Malaysia	Sri Lanka	Vietnam	Others	Total
1997	18 000	60 000	43 200	18 000	4470	25 000	22 930	191 690
2000	26 385	58 000	77 500	24 000	10 670	36 000	27 535	260 090
2001	43 000	79 000	59 000	27 000	7800	56 00	36 395	308 195
2002	45 000	80 000	66 000	24 000	12 600	75 000	38 460	341 060
2003	50 000	65 000	80 000	21 000	12 660	85 000	48 500	362 160
2004	45 000	62 000	55 000	20 000	12 820	100 000	51 659	346 479
2005	35 000	70 000	35 000	19 000	14 000	90 000	31 720	294 770
2006	44 500	55 000	20 000	19 000	14 330	100 000	36 400	289 230
2007	35 000	69 000	25 000	20 000	14 640	90 000	36 400	271 040

Source: Sreekumar [2].

90 000 MT. The production capability of countries is given in Table 1.

International markets receive pepper from Vietnam, Brazil, Indonesia, India, Malaysia, Sri Lanka. India contributes around 10% to the world market.

The productivity of black pepper is slowly declining, mainly because of the prevalence of epidemic diseases, pests and drought. Viruses, such as piper yellow mottle virus (PYMoV), cucumber mosaic virus (CMV) and badna virus are increasingly spreading through infected propagation material, resulting in yield losses. This results in increased use of biocides, thus contaminating the produce as well as the environment with residues of pesticides. 'Clean spices' is a concept that is catching on and this is achieved through integrated approaches for pest, disease and nutrient management involving resistant varieties, biocontrol, botanicals and organic farming. This also reduces soil degradation.

The yield reduction is also the result of unattractive prices, which reduce the farmer's incentive to care for the black pepper crop. The issues in market preferences are low price, bold berries and lower levels of pesticide residues, contamination with adulterants and aflatoxins.

Since black pepper is essentially a spice, varieties that are developed should have good quality attributes such as significant levels of oleoresins, piperine and volatile essential oil. Black pepper also has important medicinal properties. Importance is also given to caryophylline content, which is known for its antioxidant properties as well as its contribution to flavour. Chemoprofiling of black pepper accessions with gas chromatography mass spectrometry (GCMS) is being done to identify genotypes with hitherto undetected compounds for their pharmaceutical properties. The identification of newer properties adds value addition to black pepper, thus increasing the demand for its use for pharmaceutical products.

Genetic Resources

The Indian Institute of Spices Research (IISR) at Kozhikode, Kerala, maintains the world's largest collection of black pepper germplasm containing local cultivars, wild forms collected from the area of origin and related species. At present there are about 3516 accessions, consisting of 1266 wild relatives, 2062 cultivars, in addition to 9 exotic collections maintained in India. These collections were characterized and evaluated to estimate the genetic diversity for various yield and quality characters. The short-listed ones are being used directly for cultivation or as parents in breeding programmes.

Cultivar diversity (Table 2) is one of the principal components of diversity in black pepper. The cultivars are evolved directly from the wild *P. nigrum*. Natural selection and conscious selection by human endeavour for various traits have created diversity in cultivars. Over 100 black pepper cultivars are known. The cultivars are named after a specific feature of the vine such as colour or appearance of the plant (e.g. Karimunda, Vellanamban), leaf shape (e.g. Vattamundi), spike character (e.g. Kuthiravally and Aimpirian) or place of origin (Arakkulam munda, Perambramunda and Poonjaran munda) or after a person who introduced a cultivar into a particular tract (e.g. Yohannankodi and Thommankodi), etc. (Usually the words 'munda', 'mundi' or 'vally' all meaning vine, are suffixed with other words denoting feature of the vine, shape of the leaf, name of place or person.)

Breeding Objectives

In black pepper, a vegetatively propagated perennial, the major bottleneck is damage caused by fungus *Phytophthora capsici* (Leonian), nematode *Radopholus similis* and the pest *pollu* beetle (*Longitarsus nigripennis*). Resistant/tolerant sources are available in the germplasm. This, coupled with retention of good quality parameters, will help in producing high-quality black pepper with less usage of pesticides and fungicides. The most promising approach for bringing these characters into a single genotype is gene pyramiding through conventional and biotechnological methods. This, with the knowledge of the geographical region, in which high-quality market-driven organic pepper can be grown, will help India in retaining its permanent position in the pepper trade.

Thus important breeding objectives in black pepper are:

- high yield per vine (above 3 kg fresh berries per vine),
- high quality along with high yield (oleoresin above 10%),

Table 2 Cultivar diversity in black pepper

Sl. No.	Cultivar/variety	Remarks
1.	Aimpiryan	High yielding, performance excellent in higher elevations, good in quality. But late maturing, vigorous vines.
2.	Arakkulammunda	Moderate and regular bearer, medium in quality, well adapted.
3.	Balankotta	Cultivar with large droopy leaves, moderate and irregular bearing.
4.	Bilimallegesara	Moderate yielder, grown in Karnataka state.
5.	Chengannurkodi	Moderate yielder from South Kerala, medium in quality.
6.	Cheppakulamundi	Moderate yielder from Central Kerala, medium in quality.
7.	Cheriyakaniakadan	Popular in North Kerala, moderate and early bearing variety.
8.	Jeerakamundi	Cultivar with small leaves and short spikes, alternate bearing nature, small berries.
9.	Kalluvally	A promising North Kerala cultivar, good yielder, medium in quality with high dry recovery, drought-tolerant.
10.	Karimunda	Most popular cultivar suitable for most of the black pepper growing areas, high yielder and medium in quality, shade-tolerant.
11.	Kottan	A cultivar found in North Kerala, moderate in yield and medium in quality.
12.	Kottanadan	A high-yielding cultivar from South Kerala, drought-tolerant type.
13.	Kutching	A high-yielding Malaysian cultivar with medium quality.
14.	Kurimalai	A cultivar from Karnataka, moderate yielder with medium quality.
15.	Kuthiravally	A cultivar with long spikes, high yield and good quality.
16.	Kuttianikodi	A moderate yielder from Central Kerala with relatively long spikes and good spiking intensity.
17.	Malamundi	A moderate yielder, medium in quality.
18.	Malligesara	A common cultivar from Karnataka, relatively good in yield.
19.	Manjamundi	A moderate yielder from North Kerala, medium in quality.
20.	Narayakodi	Popular in South Kerala, moderate yielder with medium quality. Not easily affected by foot rot.
21.	Neelamundi	A good yielder from Central Kerala medium in quality, tolerant to *Phytophthora* infection.
22.	Nedumchola	A cultivar with small leaves and short spikes, moderate yielder.
23.	Neyyattinkaramundi	A cultivar from Central Kerala, medium in quality and yield.
24.[a]	Panchami	An improved cultivar developed as a selection from ‘Aimpiryan’, high yielder.
25.[a]	Panniyur-1	The first improved hybrid in black pepper. High yielding, popular throughout the pepper growing tracts, medium in quality with bold berries.
26.[a]	Panniyur-2	An improved cultivar, selection from open-pollinated progenies of ‘Balankotta’, high yielder and good in quality.
27.[a]	Panniyur-3	An improved hybrid with long spikes, high yield and medium in quality.
28.[a]	Panniyur-4	An improved cultivar developed as a selection from ‘Kuthiravally’. Late maturing type, high in yield and medium in quality.
29.[a]	Panniyur-5	An improved selection from Perumkodi. A good yielder with medium quality.
30.	Panniyur-6	An improved selection of Karimunda. Good yielder.
31.	Panniyur-7	An open pollinated progeny selection of Kalluvally. High yielding and good quality.
32.	Perambramunda	A cultivar from North Kerala, moderate yielder with medium quality.
33.	Perumkodi	A cultivar from Central Kerala, moderate in yield and quality.
34.	Poonjaranmunda	A cultivar originally from Central Kerala, sporadically found in gardens of North Kerala. Moderately good in yield and quality.
35.[a]	Pournami	An improved cultivar tolerant to root knot nematode. Good yielder with high quality.
36.[a]	Sreekara	An improved clonal selection from Karimunda high-yielding type with good quality.
37.[a]	Subhakara	An improved clonal selection selected from Karimunda, high-yielding type with good quality.
38.	Thevanmundi	A cultivar from Idukki district having field tolerance to foot rot disease.
39.	Thommankodi	A cultivar from central Kerala, moderately good in yield and quality.
40.	Thulamundi	A Central Kerala cultivar, medium in yield and quality.
41	Uddagara	A popular cultivar of Karnataka, good in yield and medium in quality.
42.	Vadakkan	A cultivar from North Kerala, medium in quality and yield with relatively large berries.
43.	Valliyakaniyakadan	A cultivar with larger leaves, medium in yield and quality.
44.	Vattamundi	A moderate yielder from Central Kerala.
45.	Vellanamban	Relatively moderate yielder and medium in quality characterized by the white colour of the young shoot tip.

[a]Improved variety.
Source: Ravindran *et al*. [3].

- adaptation to high altitude (above 1000 m),
- resistance to foot rot disease (*P. capsici*),
- resistance to *Fusarium* wilt (*Nectria haematococca* f. sp. *piperis*), particularly for Brazil,
- resistance to black berry (*Cephaleuros virescens*),
- resistance to anthracnose (*Colletotrichum gloeosporioides*),
- resistance to 'pollu' beetle (*L. nigripennis*),
- resistance to pepper weevil (*Lophobaris piperis*),
- resistance to nematodes (*Meloidogyne incognita* and *R. similis*) and
- resistance to drought.

Breeding Behaviour and Breeding Strategies

Black pepper is a predominantly self-pollinated (geitonogamy) perennial vine propagated by cuttings. Various degrees of protogyny are encountered in *P. nigrum*. However, the protogyny is ineffective to prevent selfing as the pendant spike is abundantly assured of pollen from the upper flowers and there will be many spikes dehiscing pollen grains simultaneously in a vine [4]. The stigma are reported to be receptive up to 10 days after exertion and small quantities of pollen are found in pollen sac even 5 or more days after dehiscence [5]. De Waard and Zeven [6] stated that positive geotropism, spatial arrangement of flowers, sequential ripening of the stigma and non-chronological dehiscence of anthers stimulate geitonogamic fertilization. Wind may aid pollen dispersal by agitating the spikes/branches. Gentry [7] reported high fruit set in a dioecious clone of black pepper with no visible staminate flowers, possibly as a result of apomixis. Indirect evidence of apomixis is observed in our studies too. Sasikumar *et al.* [4] concluded that selfing (geitonogamy) with occasional outcrossing is the predominant mode of pollination in cultivated bisexual black pepper varieties. Though self-incompatibility is ruled out in *P. nigrum*, other *Piper* spp., such as *Piper methysticum*, are not self-compatible [8]. Evidence of insect and wind pollination (ambophily) in some of the *Piper* spp. is also reported [9].

Conventional Approaches for Genetic Improvement

Clonal selection, open-pollinated progeny selection and hybridization are the major approaches followed in genetic improvement of black pepper [3, 10, 11]. Polyploidy breeding is also being attempted to increase the spectrum of variation. Viable sexual reproduction coupled with the excellent vegetative propagation techniques are the cornerstones in most of the breeding strategies followed in this perennial vine. Black pepper being heterozygous in its genetic architecture, as it has been propagated vegetatively over centuries; any breeding method should also ensure this heterozygosity. The breeding strategies followed in black pepper are described below.

Clonal Selection

Wide variability for yield and quality characters, occasionally even within a cultivar, is common in black pepper. Ibrahim *et al.* [12] studied genetic variability in black pepper cultivars. Selection within the popular clones such as Karimunda, Kuthiravally, Thevanmundi and Kottanadan has resulted in the production of superior varieties [13–16].

Sreekara and Subbakara are the two high-yielding varieties based on clonal selections from 216 accessions of the local cultivar Karimunda, conserved and characterized at IISR, Kozhikode, India. In the fifth year, the highest yield (fresh) recorded per plant was 7.5 kg (dry) in the case of Sreekara and 7.9 kg (dry) in the case of *Subhakara*.

Panniyur-4 is an improved variety from the clones of cv. Kuthiravally, based on the selection carried out by the Pepper Research Station, Pannivur, Kerala Agricultural University. PLD-2 is a selection from cv. Kottanandan by the Central Plantation Crops Research Institute Research Centre, Palode, Thiruvananthapuram.

Panchami, Pournami and IISR Thevam are three promising lines identified in the germplasm collections of the IISR. Panchami is a high-yielding line from an elite mother vine of cv. Aimpiriyan, and Pournami is cv. Ottaplackal (named after the house of the farmer from where this cultivar was collected). Panchami and Pournami have good yield-contributing characters [17]. The improved variety Pournami was tolerant to the root-knot nematode *M. incognita.* The performance of these two cultivars was much superior to the prevailing varieties [18, 19]. IISR Thevam is a germplasm selection from the local cultivar Thevanmudy, which has shown field tolerance to *Phytophthora* foot rot disease coupled with high yield [16].

Semangok Perak is an improved variety developed by on selection method from the introductions in Sarawak, Malaysia [20]. This variety is tolerant to *Phytopthora* foot AQ3
rot disease, as well as being a high yielder.

Open-pollinated Progeny Selection

Selections from open-pollinated seedlings of popular varieties have also yielded very good segregants. Even though the mode of pollination in black pepper is geitonogamy, there is a certain percentage of crossing and it is possible to locate useful genotypes in open-pollinated progenies. In Sarawak, Malaysia, Sim [21] identified five promising genotypes from the third generation open-pollinated progenies of the cultivars Balankotta, Cheriyakaniyakkadan and Kalluvally, which were introduced from India to Malaysia. Three black pepper varieties, Panniyur 2, Panniyur 5 and IISR Sakthi are released, so far, through open-pollinated progeny selection.

Hybridization

Genetic improvement of black pepper through hybridization involves three steps: selection of parents, developing progenies from the parents and selection of superior varieties from the progenies and their clonal multiplication. The Pepper Research Station, Panniyur was the pioneering establishment to start black pepper breeding in India, way back in the 1950s. Many intervarietal hybrids were produced, involving popular varieties as parents, and they have been evaluated for yield and quality attributes. So far, four hybrids, Panniyur-1, Panniyur-3, IISR Girimunda and IISR Malabar Excel have been released for cultivation. The important characters of these varieties are given in Table 3.

Sasikumar *et al.* [23] reported the first successful production of interspecific hybrids from the crosses of *P. nigrum*×*Piper attenuatum* and *P. nigrum*×*Piper barberi*. The hybrids were distinct in anatomical and morphological features, with chromosome number 2*n*=26. Isozyme studies revealed hybrid-specific as well as male-parent-specific bands. Recently, Vanaja *et al.* [24] developed a partly fertile interspecific hybrid resistant to *Phytopthora* foot rot disease using a cultivated *P. nigrum* and *Piper colubrinum* (an exotic wild species resistant to foot rot disease), as parents. Morphological, anatomical and molecular studies confirmed their hybridity and this hybrid can be considered as a successful breakthrough for introgression of resistance to the cultivated species.

Polyploidy Breeding

Black pepper is diploid (2*n*=52) [25]. An induced tetraploid (2*n*=4*x*=104) of the black pepper hybrid, Panniyur-1, was developed at IISR by treating fresh seeds of Panniyur-1 with 0.05% colchicine [26]. Nair *et al.* [27] reported a natural triploid (2*n*=78) among the black pepper cultivars. This triploid cultivar is characterized by very bold berries, loose setting and large leaves. Progenies of this triploid exhibit wide variation for somatic chromosome number and morphology [26, 27].

The varieties/hybrids developed/released through the above strategies in India and their salient features are given in Table 3.

The improved black pepper varieties of Malaysia are Semongok Perak (selection) and Semongk Emas (back-cross progeny of Balankotta×Kutching); Indonesia are Natar-1 and Natar-2 (both germplasm selections), while that of Sri Lanka is PW 14 and of Madagascar are Sel. IV.1 and Sel. IV.2 (both selections) [28].

Kutching is the most extensively grown cultivar in Malaysia, though susceptible to *Phytophthora* foot rot and black berry, as compared with the two new varieties that are early bearing and resistant to black berry. Semongok Perak, though early in yielding, does not sustain the yield after the third crop as it gets infected with pepper weevil [29].

Kutching, known as Chingapura in Brazil, was also the leading black pepper cultivar in Brazil until 1982. However, since 1982, two new cultivars, Bragantina (a ‘clonal selection’ of Panniyur-1 hybrid) and Guajarina (a clonal selection of the Indian cultivar Arakulam munda), have become popular in Brazil. Clonal variability in Panniyur-1 is reported from India too [30]. However, Shahnas *et al.* [31] failed to observe any major polymorphic DNA bands in the different nodal cuttings of Panniyur-1 and Sreekara in a random amplified polymorphic DNA (RAPD) study using 10 random decamer primers barring a single polymorphic band each produced by two primers in the bottom nodal cuttings of Sreekara (in a nursery study). Guajarina is superior to Bragantina as it is more tolerant to water stress and is of good quality. All the Brazilian cultivars are bisexual and exhibit variability for spike length, size and number of berries and setting percentage besides for the morphological traits [32]. *Fusarium* wilt is a serious disease of black pepper caused by *N. haematococca* f. sp. *piperis* in Brazil and berry spot caused by *C. virescens* is a serious field disease in Brazil, Malaysia and Indonesia. Anthracnose caused by *C. gloeosporioides* is a common leaf and spike disease of black pepper in India and Brazil. In Brazil, Guajarina was the least susceptible (29.5% infestation) to *C. gloeosporioides* and the most resistant to leaf fall (15.07%) [33].

Breeding for Nematode, Disease and Insect Resistance

Phytophthora foot rot (quick wilt) is the major disease of black pepper in all pepper growing countries, resulting in heavy crop losses. The new varieties IISR Thevam and IISR Shakti are highly tolerant to foot rot disease.

The burrowing nematode (*R. similis*) and root-knot nematode (*M. incognita*) are serious problems in some black pepper tracts. A root-knot-nematode-tolerant cultivar, Pournami, has been developed through germplasm selection.

Pollu beetle is a major insect pest of black pepper in India. At IISR, evaluation of the germplasm accessions resulted in the identification of four accessions of cultivated black pepper: Accession Nos. 816, 841, 1084 and 1114 to be relatively resistant to *pollu* beetle. Incorporation of *pollu* beetle resistance through interspecific hybridization involving *P. attenuatum* and *P. barberi* with *P. nigrum* has been achieved [23]. Pepper weevil (*L. piperis*) is an important pest of black pepper in Brazil and Malaysia and mealy bug (*Pseudococcus* sp.) is a major root pest, mainly in India and Brazil.

Table 3 Improved varieties of black pepper from India and their salient features

Name	Pedigree	Released from	Average yield dry (kg/ha)	Oleoresin (%)	Piperine (%)	E. oil (%)	Remark
Panniyur-1	F_1 of Uthirankotta ×Cheriyakaniyakadan	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	1242.0	11.8	5.3	3.5	Suited to all pepper growing regions. Not suited to heavily shaded areas.
Panniyur-2	Open-pollinated progeny selection of Balankotta	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	2570.0	10.9	6.6	3.4	Reported to be shade tolerant.
Panniyur-3	F_1 of Uthirankotta ×Cheriyakaniyakadan	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	1953.0	12.7	5.2	3.1	Late maturing. Suited to all pepper growing regions.
Panniyur-4	Clonal selection from Kuthiravally	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	1277.0	9.2	4.4	2.1	Stable yielder.
Panniyur-5	Open-pollinated progeny selection of Perumkodi	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	1098.0	12.3	5.5	3.8	Tolerant to nursery diseases and shade.
Panniyur-6	Clonal selection of Karimunda	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	2127.0	8.3	4.9	1.3	Suited to all pepper tracts.
Panniyur-7	Open-pollinated progeny of Kalluvally	Pepper Research Station, Panniyur, Kerala Agricultural University (KAU), Kannur, Kerala, India.	1410.0	10.6	5.6	1.5	Suited to all pepper tracts.
Subhakara	Clonal selection from Karimunda	Indian Institute of Spices Research, P.O. Marikunnu Kozhikode, Kerala, India.	2352.0	12.4	3.4	6.0	Suited to all pepper growing regions.
Sreekara	Clonal selection from Karimunda	Indian Institute of Spices Research, P.O. Marikunnu Kozhikode, Kerala, India.	2677.0	13.0	5.1	7.0	Suited to all pepper growing regions.
Panchami	Germplasm selection	Indian Institute of Spices Research, P.O. Marikunnu Kozhikode, Kerala, India.	2828.0	12.5	4.7	3.4	Late maturing type suited to all pepper growing areas.
Pournami	Germplasm selection	Indian Institute of Spices Research, P.O. Marikunnu Kozhikode, Kerala, India.	2333.0	13.8	4.1	3.4	Tolerant to root knot nematode (*M. incognita*).
PLD-2	Clonal selection from Kottanadan	IISR, Kozhikode and Central Plantation Crops Research Institute, (CPCRI), Regional Station, Palode, Kerala.	2475.0	15.5	3.3	3.5	Suited to Trivandrum and Quilon districts of Kerala, India.
IISR Thevam	Clonal selection of Thevanmundi	IISR Kozhikode	1787.0	8.15	1.6	3.1	Tolerant to foot rot disease (durable resistance). Suited to high altitudes and plains.
IISR Malabar Excel	F_1 of Cholamundi ×Panniyur-1	IISR Kozhikode	1065.0	13.5	2.96	3.2	Suited to high altitudes and rich in oleoresin.
IISR Girimunda	F_1 of Narayakodi ×Neelamundi	IISR Kozhikode	2112.0	9.65	2.2	3.4	Suited to high altitudes
IISR Sakthi	Open-pollinated progeny of Perambramundi	IISR Kozhikode	5.17**	10.2	3.3	3.7	Tolerant to foot rot disease in the juvenile phase

Source: Parthasarathy [22].

Breeding for Drought Tolerance

Screening of germplasm against moisture stress has resulted in identifying few promising Karimunda lines: KS 69, KS 51 and KS 114, Accession Nos. 813, 931 and 1495. These drought-tolerant lines are undergoing yield evaluation.

Breeding for High Altitude

Black pepper grows well in altitudes up to 3000 ft above mean sea level (MSL). Most of the black pepper varieties are not specifically bred for high altitudes, where it can be grown either as a monocrop or as an intercrop on the shade trees of tea or coffee or cardamom estates. Evaluation of 100 hybrids at Valparai (3000 ft above MSL) has helped in identifying two hybrids, HP-813 and HP-105, which were released as IISR Malabar Excel and IISR Girimunda, respectively. IISR Thevam is another variety performing well at higher altitudes. The identified lines with resistance to biotic and abiotic stresses were involved in breeding programmes to bring these characters into a single genotype.

Breeding for High Quality

Black pepper cultivars rich in piperine, oleoresin and essential oil are in great demand in the value-added produce industry. Screening of the germplasm has resulted in identifying some cultivars with high piperine, oleoresin and oil content. The Indian cultivars Kottanadan, Kumbakodi, Kuthiravally and Nilgiri are rich in piperine and oleoresin, whereas Balankotta, Kaniyakadan and Kumbakody are rich in essential oil. Sreekara and Subhakara varieties have high oil content (>6%). Zachariah [34] observed variability for essential oil and major oil constituents in 42 accessions of black pepper. Gopalam and Ravindran [35] also reported variability for quality traits in black pepper cultivars. These high-quality lines are being used in a hybridization programme to develop high-quality, high-yielding cultivars. IISR Malabar Excel (HP 813) [16] and PLD 2 are improved hybrids/varieties rich in oleoresin (above 12%).

Five varieties of black pepper (*P. nigrum*), Panniyur-1, Panniyur-2, Panniyur-5, Sreekara and Subhakara, were graded in a hand-operated rotary sieve cleaner-cum-grader with pore size of 3.5, 3.8 and 4.8 mm placed one after the other in increasing order. The variety Subhakara had the highest amount of berries of size between 3.8 and 4.8 mm (33.3%), which represents the Tellicherry Garbled Special Extra Bold (TGSEB) grade. Panniyur-1, Panniyur-2 and Panniyur-5 had more than 60% of their berries under the grade Tellicherry Garbled (TG). Bulk density ranged from 450 to 571 g/l. Bulk density increased with an increase in size. However, bulk density was found to decrease when the berry size was above 4.8 mm. The primary metabolites such as starch increased as the grade size increased, whereas protein and crude fibre did not show any consistent trend. The secondary metabolites, such as oleoresin and piperine content, were highest for the lower grade (<3.5 mm), but the essential oil content did not vary with respect to grade in all the varieties [36].

Utpala *et al.* [37] have done geographic information studies (GIS) of the species of Western Ghats and reported that low-altitude species have considerable morphological distance from the high-altitude species in a dendrogram drawn with morphological characters. *P. nigrum* is found in a vast altitudinal diversity, and shows great adaptability to a wide range of climatic and soil conditions, which leads to interspecies diversity. *P. nigrum* collected from the Western Ghats of Karnataka and Kerala were studied for the leaf volatile oil with the help of gas chromatography and the results are plotted in a map with the help of Arc-GIS software to understand the influence of location. Though 7–15 compounds were detected from volatile oils in different accessions, maximum variability was observed with respect to β-caryophyllene and nerolidol in the leaf oil of *P. nigrum* and the influence of location on these components is significant [37].

The composition of essential oil of 17 cultivars of Kerala indicates the presence of 69.4–85.0% monoterpene hydrocarbons and 15.0–27.6% sesquiterpene hydrocarbons and the rest were oxygenated constituents. With regard to the major monoterpene hydrocarbons, α-pinene ranged from 5.9 to 12.8%, β-pinene from 10.6 to 35.5% and limonene from 22.0 to 31.1%. The major sesquiterpene hydrocarbon, β-caryophyllene ranged from 10.3 to 22.4% [38]. Analysis of four new genotypes of pepper (Panniyur-1, Panniyur-2, Panniyur-3 and Panniyur-4) by a combination of GCMS and Kovats indices on a methyl silicone capillary column revealed that the three Panniyur genotypes contained α-pinene in the range of 5.07–6.18%, β-pinene (9.16–11.08%), sabinene (8.50–17.16%), limonene (21.06–22.71%) and β-caryophyllene (21.57–27.70%). The oil from Panniyur-4 contained α-pinene (5.32%), β-pinene (6.40%), sabinene (1.94%), myrcene (8.40%), *p*-cymene (9.70%), limonene (16.74%) and caryophyllene (21.19%) [39]. Zachariah *et al.* [40] conducted a study on the effect of grafting *P. nigrum* on *P. colubrinum* as rootstock. The cultivars used for grafting are Panniyur-I, -II, -III, -IV and -V, Malligesara, Pournami, Sreekara, Poonjaranmunda, Kuthiravally and Balankotta. The major essential oil constituents in grafts and non-grafts of pepper cultivars were pinene, sabinene and β-caryophyllene. Caryophyllene content varied from 12 to 27% in graft and 7 to 29% in non-graft. Limonene content varied from 13 to 24% in graft and 13 to 22% in non-graft.

Variation of Piperine in Relation to Cultivars

Seven black pepper cultivars, namely Panniyur-2, Panniyur-3, Panniyur-4, Sreekara, Subhakara, KS-88 and

Table 4 Black pepper accessions short listed for breeding objectives

Sl. No	Trait	Germplasm accessions short listed
1	Tolerance/resistance to foot rot disease	IISR, Thevam, IISR Shakti
2.	Resistance to *pollu* beetle	Accession Nos. 816 (Neyyatinkara mundi), 841 (Veluthakaniyakadan), 1084 (Cheppukulamundi) and 1114 (Kumbhachola).
3.	Tolerance/resistance to slow wilt (nematodes)	Pournami, Accession Nos. 820 (Perumkodi) & Hybrid 39 (Irumaniyan × Karimunda)
4	Tolerance to drought	Karimunda selections (KS) 69, KS 51 and KS 114, 813 (Ottaplackal-II), 931 (Kalluvally) and 1495 (Kottanadan).
6	High caryophyllene content	Accession Nos. 840 (Vattamundi), 971 (Balankotta), 1019 (Vellamunda) and 1022 (Karimunda).

Neelamundi (a local cultivar), were evaluated for piperine, oleoresin and essential oil contents. Panniyur-4 recorded the lowest oleoresin (9.2%) and essential oil contents (2.1%) and relatively medium piperine content (4.4%). Panniyur-2 had poor yield but recorded the highest piperine content (6.6%). Neelamundi, KS-88 and Sreekara gave the highest oleoresin contents (13.9, 13.1 and 13.0%, respectively), while Sreekara and Subhakara gave the highest essential oil content (7.0 and 6.0% respectively). HP-813 (IISR Malabar Excel) had reportedly high oleoresin and piperine content compared with other recently released black pepper cultivars. Five different grades of four (*P. nigrum*) cultivars were analysed for their piperine percentage, oleoresin and volatile oil content. The garbled light special (GL special) grade of each cultivar had the highest piperine percentages. The TG grade of Kalluvally had the highest volatile oil content of 3.4%. Panniyur-6 is a high-yielding clone with 8.27% oleoresin, 4.94% piperine and 1.33% volatile oil content. Panniyur-7, a seedling progeny of Kalluvally type, a superior open-pollinated progeny, has 10.61% oleoresin, 5.57% piperine and 1.50% volatile oil content [39].

Grafting with Resistant Root Stocks

Phytophthora foot rot and slow decline are the major maladies for black pepper. To overcome this, *P. colubrinum*, an exotic wild species of *Piper* is effectively utilized as a rootstock for grafting black pepper to control foot rot and nematodes in addition to adaptability to marshy situations. The plant has been found to be immune to the foot-rot-causing fungus *P. capsici* and resistant to nematodes that cause root knots in black pepper. Five-month-old seedlings or rooted cuttings of *P. colubrinum* are used as rootstock. Grafting at 50 cm height is needed to avoid splashing of soil and debris that contain fungal spores to the pepper vine and also to have more aerial roots growing into the ground. Several methods of grafting were tried and the best performance was seen with the double rootstock method. Shoots taken from runner vines trailing on the ground that arise from mature vines were used as scions. Each scion has either a node with a leaf or 2–3 nodes without leaves. The ideal time for grafting is during rains. Sprouting is observed 20 days after grafting and the union is complete after three months when the plastic wrapping around graft union can be removed. A yield of about 1.0 kg dry/graft can be obtained by the third year from the grafts. The evaluation of grafts in the field indicated that they remained healthy even nine years after planting. The rootstock did not have any influence on qualities of the berries.

IISR Initiatives towards Developing Varieties Resistant to Biotic and Abiotic Stresses

Black pepper hybrids/varieties combining disease and pest resistance along with high yield and high quality increases the productivity. Field screening of black pepper germplasm resulted in the identification of resistant/tolerant lines against various biotic and abiotic stresses (Table 4). However, most of these accessions are not high yielders. Efforts are initiated to transfer these features to improved cultivars through intercultivar hybridization using the improved variety Subhakara as a female parent.

Registration of Germplasm

Registration of valuable and important germplasm meets the requirement of intellectual property rights and released issues, breeders' rights, farmers' rights, etc. In this regard, IISR Calicut has registered four unique black pepper germplasm (Table 5) with unique characters.

Biotechnological Approaches

Application of biotechnological tools will be of immense help in the improvement of spices, especially in black pepper. AQ4

Micropropagation

In vitro propagation of black pepper has been reported using shoot tips, nodal segments and apical meristems. Multiple shoots can be induced using BA in the culture AQ5

Table 5 List of germplasm registered

Sl. No	Crop	Year	Reg. No	Coll. No	Traits
1	Black pepper	2003	INGR 03091	1041	Field tolerance to foot rot disease
2	Black pepper	2004	INGR 370011	5455	High oleoresin and bold berries
3	Black pepper	2006	INGR 06026	1019	High caryophyllene
4	Black pepper	2008	INGR 8100	IC 563950	Novel spike variant with 100% proliferating spikes

medium either alone or in combination with auxins. *In-vitro*-developed shoots could be easily rooted using growth-regulator-free basal medium [41, 42].

Micropropagation coupled with virus indexing and genetic fidelity testing is a good technology for obtaining disease and virus-free planting materials. In India, tissue-cultured black pepper plants fortified with biocontrol agents such as versicular arbuscular mycorrhiza (VAM), *Trichoderma viride* and plant growth-promoting rhizobacteria (PGPR) were evaluated in over 100 ha in comparison with rooted cuttings and the tissue-cultured plants showed better establishment, better growth, early flowering and lower disease incidence than controls.

Protocols for plant regeneration were standardized and plants were regenerated from shoot- and leaf-derived callus cultures [43–46]. The plants could be established in the field. Micropropagation through direct somatic embryogenesis and subsequent cyclic secondary somatic embryogenesis was developed by Nair *et al.* [15, 47].

Protoplast Culture

Shaji *et al.* [48] reported high-frequency isolation of viable protoplasts from *in-vitro*-derived leaves of *P. nigrum* and *P. colubrinum*. Regeneration and development of protoclones were observed only in *P. colubrinum* protoplast cultures.

Genetic Transformation

Preliminary reports are available on the *Agrobacterium*-mediated gene transfer system in *P. nigrum* [49]. Obtained primary transformants for kanamycin resistance in the cotyledons using *A. tumefaciens* binary vector strains AQ6 LBA4404 and EHA105. Sim *et al.* [50] cultured explants from leaf, petiole and stem explants from axenic seedlings of black pepper inoculated with the *A. tumefaciens* strain LBA4404 containing vectors carrying the *nptII* and *gus* [*uidA*] genes in callus inducing medium in the dark at 28°C. At 10 days after inoculation with *A. tumefaciens*, explants AQ7 were sampled for GUS expression by X-gluc staining. Expression of the *gus* gene confirmed gene transfer to recipient tissue.

Molecular Markers

Molecular profiling of important cultivars, released varieties and related species were done to study their interrelationships. Molecular markers were also used to study the genetic similarity/variability of major accessions, hybrids and their parents. Marker-aided studies for screening genetic stability in micropropagated plants of *Piper* have been reported.

Pradeep Kumar *et al.* [30, 51] studied 24 black pepper (*P. nigrum*) accessions, including nine advanced cultivars and 13 landraces using RAPD markers. Good variability was observed among *P. nigrum* cultivars. Genetic proximity among *P. nigrum* cultivars could be related to their phenotypic similarities or geographical distribution. Greater divergence was observed among landraces than among advanced cultivars. Cultivar-specific bands were obtained for all cultivars tested except for Panniyur-3. An assessment of genetic relationships among 30 popular and agronomically important cultivars of black pepper using amplified fragment length polymorphism (AFLP) analysis was reported by Nisha *et al.* [52]. The dendrogram derived by unweighted pair group method analysis (UPGMA) grouped the accessions into three major clusters and four diverse cultivars with only 30% similarity. Karimunda, a widely grown and popular cultivar was unique in the fingerprint profiles obtained.

Nazeem *et al.* [53] used both RAPD and AFLP analysis to assess the variability and interrelationships among 49 cultivars of black pepper. Two selections from the variety Karimunda, namely Sreekara and Subhakara, together formed a single cluster with almost 92% similarity. However, Panniyur 1 and Panniyur 3, which are the progenies of the same parentage were distinctly dissimilar. Sreedevi *et al.* [54] used RAPD to characterize seven new high-yielding lines of black pepper and developed a bar code to identify the varieties.

Nirmal Babu *et al.* [55, 56] used morphological characters coupled with RAPD, ISSR profiles to estimate the genetic fidelity of micropropagated plants pepper cv. Subhakara and Aimpiriyan. They observed that the micropropagated plants are genetically stable and the micropropagation technology can be used for commercial cloning of black pepper.

Gene Isolation

Efforts are being made to discover plant genes that are up-regulated during resistant interactions between *P. colubrinum* and *P. capsici*. Differential display reverse transcriptase polymerase chain reaction (RT-PCR) was carried out using RNAs isolated from *P. colubrinum* (resistant to *Phytophthora*) to tag genes expressed on *P. colubrinum* in response to *Phytophthora* inoculation (unpublished results). Two cDNAs corresponding to differentially expressed genes in *P. colubrinum* were cloned and the $3'$ end of the gene was sequenced.

Conclusion

The global demands for black pepper are cheaper produce with bold berries and high quality, with lower pesticide residues. All these are not possible without developing high-yielding, high-quality varieties with resistance to biotic and abiotic stresses. Development of short-duration varieties with increased medicinal properties and reduced side effects will add value to black pepper, offering attractive prices and thus increasing the income of pepper farmers. Gene pyramiding, though a long process, will help in bringing desired characters distributed in the germplasm into a single genotype. This is an important approach which must be perused with vigour. Production of good-quality disease-free planting material is another important input. Development of diagnostic markers for detecting biotic stress, tagging agronomically important characters will help in marker-assisted selection and reduce breeding time and help in genetic fidelity testing.

During the past decade, the production and productivity of black pepper has increased substantially. The year 2005–2006 witnessed the highest productivity: 357 kg/ha. Even though the area under pepper cultivation has slightly reduced after 1998–1999, the production and productivity has increased substantially. This indicates that farmers are giving better care to their existing plantations to increase the yield. However, productivity reduced to 281 kg/ha during 2006–2007 and the area has also come down. The productivity can be increased further by popularizing the high-yielding improved varieties, replacing the old senile cultivar/varieties and popularizing the adoption of latest technologies in crop management practices.

White pepper constituted 16.9% of total export of pepper, whereas India's contribution to white pepper production and export is insignificant.

The major thrust in future research programmes should be oriented towards the following focused approaches for increasing the production and productivity of black pepper:

- Conservation of genetic resources and bar-coding of genotypes, in order to have complete information on gene pool which is native to India for sustainable utilization and conservation.
- Increasing the productivity of spices, so as to raise the production levels by using improved varieties with high yield, quality traits and disease/pest resistance. AQ8
- Development of diagnostic markers for detecting biotic stresses: this will help in marker-assisted selection and reduce breeding time and help in genetic fidelity testing.

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