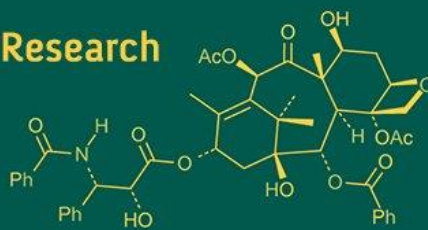


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Resilience in the Rainfed tropics: Identifying drought-tolerant black pepper germplasm for climate-adaptive spice farming

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Abstract

Black pepper (*Piper nigrum* L.), a major spice crop of economic importance, is highly vulnerable to climate variability, particularly drought stress in rainfed production systems. The present study was conducted from 2020 to 2023 to evaluate genetic variability for drought tolerance and to identify resilient genotypes for future breeding programs. A total of 36 genotypes were screened under controlled pot culture conditions using six-month-old rooted cuttings. Drought stress was imposed by withholding irrigation for 15 days, and observations were recorded at 5, 10, and 15 days after stress induction. Key physiological and biochemical parameters, including relative water content (RWC), membrane leakage, proline content, total chlorophyll, and antioxidant enzyme activities (peroxidase, polyphenol oxidase, and glutathione reductase), were assessed. The results revealed significant variation among genotypes in response to drought stress. A consistent decline in RWC and chlorophyll content, along with increased membrane leakage, was observed with increasing stress duration, indicating progressive cellular damage. In contrast, proline accumulation and antioxidant enzyme activities increased under stress, reflecting adaptive responses to oxidative damage. Genotypes maintaining higher RWC and lower membrane leakage exhibited better drought tolerance, suggesting these traits as reliable selection indices. Certain genotypes demonstrated superior performance across multiple parameters, indicating their potential as drought-resilient candidates. The study confirms that drought tolerance in black pepper is a complex trait governed by multiple physiological and biochemical mechanisms. The identified tolerant genotypes provide valuable genetic resources for crop improvement programs aimed at developing climate-resilient varieties. Integrating physiological screening with biochemical markers offers an effective approach for early-stage selection of drought-tolerant genotypes. These findings contribute to sustainable black pepper production under changing climatic conditions and highlight the importance of targeted breeding strategies to enhance resilience in spice crops.

Keywords: Black pepper, drought, relative water content, climate resilience

1. Introduction

Black pepper (*Piper nigrum* L.), known as the “King of Spices” or “Black Gold,” is a major commercial spice in India. Global production reached 1,103,024 MT in 2021, with India ranking fourth (61,004 MT) (FAO, 2020) [6]. Although India accounts for 42.55% of the global area, its production share is only 16.76%. Kerala contributes 80-90% of national output, with Idukki and Wayanad together covering 64.21% of area and 76.16% of production.

Climate variability has significantly affected productivity. A long-term analysis (1840-2014) showed Kerala’s average rainfall (2817±406 mm) follows a cyclic trend, with declining annual and southwest monsoon rainfall but increasing post-monsoon rainfall in recent decades (Kandiannan *et al.*, 2014) [10]. These shifts have contributed to yield decline. Low productivity is further linked to poor genetic potential, senile vines, biotic and abiotic stresses, lack of quality planting material, and suboptimal agronomic practices.

Climate change remains a key constraint, particularly for smallholders. Developing climate-resilient varieties tolerant to heat, drought, flooding, salinity, and cold is essential. Among abiotic stresses, drought is most critical in Kerala’s rainfed systems, though recent floods have also caused damage (Maheswari *et al.*, 2012) [12]. Stress tolerance depends on a plant’s ability to survive, reproduce, and recover with high productivity after stress.

These traits are quantitatively inherited and governed by multiple loci (Wu *et al.*, 2011) ^[19], making their genetic understanding crucial. Adaptation strategies include moisture conservation, water harvesting, residue management, conservation agriculture, site-specific nutrient management, and cultivation of stress-tolerant varieties. Developing and disseminating such cultivars remains an urgent priority to sustain black pepper production under changing climatic conditions (Maheswari *et al.*, 2012; Shanker *et al.*, 2014) ^[12].

The study was conducted at the Farming Systems Research Station from 2020 to 2023 to evaluate germplasm for drought tolerance under controlled conditions and to identify resistant genotypes for future research.

2. Materials and Methods

Thirty-six genotypes collected from different parts of south Kerala (Thiruvananthapuram, Kollam and Pathanamthitta) were assessed for drought tolerance under controlled pot culture conditions using six-month-old rooted cuttings. Drought stress was induced by withholding irrigation for 15 days, and observations were recorded at 5, 10, and 15 days after stress induction (DASI). Parameters evaluated included soil moisture, relative water content (RWC), membrane leakage, proline, total chlorophyll, peroxidase, polyphenol oxidase, and glutathione reductase. Irrigated plants served as controls, and the third fully expanded leaf was used for analysis.

2.1 Soil moisture content (%): Soil moisture was estimated using the gravimetric method. Fresh soil samples collected from the root zone were weighed, then oven-dried at 180 °C until a constant weight was achieved. Moisture content was calculated as:

$$\text{Moisture percent by weight} = [(M_1 - M_2) / (M_1 - M)] \times 100$$

M = weight of empty dish, M_1 = Fresh weight of soil, M_2 = weight of dish with dry soil

2.2 Relative water content (RWC) (%): RWC was determined following Barrs and Weatherly (1962). Leaf samples were cut into small pieces, and fresh weight was recorded. After soaking in distilled water for 4 hours, turgid weight was measured. Samples were then oven-dried at 80 °C to obtain dry weight.

RWC can be calculated using the following equation:

$$\text{RWC (\%)} = (\text{Leaf fresh wt.} - \text{Leaf dry weight}) / (\text{Leaf turgid weight} - \text{Leaf dry weight}) \times 100$$

2.3 Membrane Leakage (%): Membrane leakage was measured based on electrical conductivity. Leaf samples were incubated in distilled water, and initial conductivity (EC_1) was recorded. After boiling and cooling, final conductivity (EC_2) was measured.

Membrane leakage was calculated using the following equation:

$$\text{Membrane Leakage} = (EC_1 / EC_2) \times 100$$

EC_1 -initial EC, EC_2 -Final EC after boiling.

2.4 Proline (μ mol/g): Proline was estimated by homogenizing plant tissue in 3% sulphosalicylic acid, followed by centrifugation. The extract was reacted with glacial acetic acid and acid ninhydrin, heated, and then extracted with toluene. Absorbance was recorded at 520 nm, and proline concentration was calculated using a standard curve.

$$\text{Proline content (\mu mol/g)} = \mu \text{ proline/ml} \times \text{ml toluene} / 115.5 (\text{5/g sample})$$

2.5 Chlorophyll (mg g⁻¹): Chlorophyll content was determined using the Arnon (1949) method. Leaf samples were extracted in 80% acetone: DMSO (1:1), and absorbance was recorded at 645 and 663 nm.

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times 1 \times V / (1000 \times W)$$

- Absorbance at specific wavelength
- V-Final volume of chlorophyll extract in 80% acetone: DMSO mixture (25 ml)
- W-Fresh weight of tissue extracted

2.6 Estimation of Peroxidase (activity/min/g): Peroxidase activity was estimated following Srivastava (1987). Enzyme extract was prepared in phosphate buffer, and activity was measured using pyrogallol and hydrogen peroxide by recording changes in absorbance at 420 nm.

$$\text{Peroxidase activity (activity/minute/g)} = (\text{difference in absorbance in 60 seconds} / 50) \times 5000$$

2.7 Estimation of polyphenol oxidase (activity/min/g): Polyphenol oxidase activity was determined using the method of Mayer *et al.* (1965). The reaction mixture contained phosphate buffer and enzyme extract, and the reaction was initiated with catechol. Absorbance was recorded at 495 nm.

$$\text{Polyphenol oxidase activity} = (\text{difference in absorbance in 60 seconds} / 50) \times 5000$$

2.8 Estimation of Glutathione reductase (activity/min/g): Glutathione reductase activity was measured by homogenizing leaf tissue in phosphate buffer with EDTA, followed by centrifugation. The reaction mixture included NADPH and oxidized glutathione, and the decrease in absorbance at 340 nm was recorded.

1 unit of enzyme activity = the amount of enzyme that catalyses the reduction of 1 μ mol of NADPH/minute.

$$\text{N moles of NADPH/ml} = (\text{difference in absorbance at 340/minute}) / 6.22 \times 10^{-3} \mu \text{ m}^{-1}$$

3. Results and Discussion

The collected varieties were screened for drought tolerance in pot culture study. Seven characters including physiological and biochemical parameters were recorded. Results are presented in table 1 to 7. Soil moisture was recorded for every observation and is presented in table 8. Drought tolerance is a complex, quantitatively inherited trait governed by multiple genes, making it difficult to analyze

and characterize. Plants adopt diverse strategies to survive water deficit, such as enhancing root growth to improve water uptake and reducing transpiration to limit water loss. They may also employ combined adaptive mechanisms, including the production of protective proteins that maintain membrane stability and cellular function. Additionally, osmolytes like proline, sorbitol, and glycine betaine accumulate in vacuoles, helping reduce cellular water loss.

A clear understanding of biochemical and molecular responses to drought is essential for elucidating resistance mechanisms. Drought stress affects plant growth, yield, membrane integrity, pigment content, osmotic balance, water relations, and photosynthesis (Benjamin and Nielsen, 2006) ^[4]. The degree of susceptibility varies depending on stress intensity, associated environmental factors, species, and developmental stage (Demirevska *et al.*, 2009) ^[5]. Plant acclimation to water deficit involves structural, physiological, and biochemical adjustments, including changes in growth rate, osmotic potential, and antioxidant defense systems. Therefore, improving drought resistance in crops is critical to maintaining productivity under adverse conditions.

The development of climate-resilient crop varieties with tolerance to multiple stresses such as drought, heat, salinity, cold, and flooding is essential for sustaining yields under climate change. Among these, drought remains a major constraint to plant growth and productivity (Maheswari *et al.*, 2012) ^[12]. In Kerala, drought is the primary abiotic stress affecting pepper cultivation, although recent floods have also caused significant damage. Identifying key traits that support plant growth and survival under stress is crucial for developing tolerant cultivars.

3.1 Screening of germplasm for drought under artificial conditions

The collected varieties were screened for drought tolerance in pot culture study. Seven characters including physiological and biochemical parameters were recorded. Mean values are presented in table 4. Mean soil moisture was recorded for every observation and is presented in table 5.

3.1.1 Relative water content (%) of black pepper genotypes

The availability of water is the major factor influencing the growth, development and productivity. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues. It is used as an index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf and reduces as the dry matter accumulates. A decrease in the RWC in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006) ^[13].

The relative water content is high for all the genotypes under control when compared to the stress induced ones (Table 1). In five days after stress induction the relative water content ranged from 43.35% (Cholakkodi) to 85.74 (Sadanandapuram local). The mean value for the control is 83.97% and for five days after stress induction is 70.81%. For ten days after stress induction the RWC ranged from 35.86% (Cholakkodi) to 85.04% (Sadanandapuram local). The mean value for control is 81.50% and for ten days after stress induction is 61.44%. For fifteen days after stress induction the RWC ranged from 30.19% (Cholakkodi) to

68.37% (Sadanandapuram local). The mean value for control is 78.32% and for fifteen days after stress induction is 46.16%.

Comparatively, higher RWC and low membrane leakage are associated with drought tolerant genotypes. Thankamani and Ashokan (2004) ^[17] reported that in all the varieties tested the maximum relative water content was observed in plants without water stress. The daily irrigated plants of the varieties Kalluvally-1, Kalluvally-2, Panniyur-4 recorded RWC above 90 percent. A stable RWC under the stress may be considered as one of the indices for screening water stress tolerant varieties. Earlier reports also showed very good correlation between RWC and water stress tolerance.

3.1.2 Membrane leakage (%) of black pepper genotypes

The electrolyte leakage technique to evaluate the cell membrane stability as a mechanism of water stress tolerance in plants. Relative water content and membrane integrity were reduced with increasing stress intensity. Relative water content was positively correlated with membrane integrity and negatively correlated with stress intensity.

The Angamaly (PRS-64) showed highest relative water content, membrane integrity which was on par with Panniyur-5 (PRS-115), PRS-160, Panniyur-8 (PRS-153) (Table 2). The lowest relative content, membrane integrity observed in Panniyur-1 (PRS-44) which was par on Karimunda (Pallavi, 2015) ^[14]. In five days after stress induction membrane leakage ranged from 20.13 (Arikkilazhi) to 66.78 (Padappan). The mean value for the control is 30.20% and for five days after stress induction is 46.90%. For ten days after stress induction the membrane leakage ranged from 38.33% (Palakkodi) to (Narayakodi). The mean value for control is 33.57% and for ten days after stress induction is 60.28%. For fifteen days after stress induction the membrane leakage ranged from 52.64% (Pandithitta local) to 88.09% (Kumbanadan). The mean value for control is 37.02% and for fifteen days after stress induction is 72.85%.

Drought is one of the major abiotic stresses affecting plants which can result in limited growth and photosynthesis. The RWC and membrane leakage of drought induced black pepper leaves were analyzed, and we have observed that the drought tolerant genotype, Acc 4216, had higher RWC and lower membrane leakage compared to the susceptible genotype, Sreekara at 10 DASI. The higher RWC and lower membrane leakage is an indicator for drought tolerance in black pepper (Krishnamurthy *et al.*, 1998) ^[11]. As these physiological parameters are multi-gene controlled traits, the variation in the traits between tolerant and susceptible black pepper genotypes may reflect the differential expression of important drought responsive genes at the molecular level. The relative expression study of differentially expressed genes can help in understanding the key genes and mechanism involved in drought tolerance in black pepper (George *et al.*, 2017) ^[7].

Thankamani and Ashokan (2004) ^[17] reported that water stress significantly influenced the membrane stability of the varieties tested. The membrane stability decreased significantly when the plants were subjected to water stress for two days; highest membrane leakage was observed in TMB1 (96.3) and lowest in Kottanadan (21.4). Only, six varieties survived after four days of water stress and the leakage was Maximum in Uthirankotta-2 (47.3) and least in Poonjarmunda (30.8). A stable RWC under the stress may

be considered as one of the indices for screening water stress tolerant varieties. Earlier reports also showed very good correlation between RWC and water stress tolerance (Balasimha *et al.*, 1988 and Vasantha, 1996) [2, 18].

Varieties which tolerated four days of water stress showed relatively less membrane leakage. Vasantha (1996) [18] imposed water stress on eight months old rooted cuttings of black pepper of fourty cultivars. Among them only six cultivars viz., Kalluvally-4, Kumbakodi, Padarpan, Panniyur-5, Poonjarmunda and Uthirankotta-2 were survived even after the fourth day of water stress. These cultivars showed highest RWC and membrane stability index. Relative water content decreased and membrane leakage increased due to water stress in different black pepper genotypes. The RWC in black pepper accessions were reduced after stress induction which ranges from 4.7 (acc 1466) to 12% (acc 1411) after 4 days of stress and from 16% (acc 1466) to 46.3% (acc 1411) after 8 days of stress. The membrane leakage increases from 35% (acc 1368) to 83.4% (acc 1409) after 4 days of stress and from 105% (acc 1368) to 253.5% (acc 1411) after 8 days of stress (Pallavi, 2015) [14].

3.1.3 Proline content (μ mol/g) of black pepper genotypes

In five days after stress induction proline content ranged from 4.24% (Cholakkodi) to 17.95% (Pandithitta local) (Table 3). The mean value for the control is 5.32% and for five days after stress induction is 10.93%. For ten days after stress induction the proline content ranged from 4.89% (Panniyur 1) to 24.57% (Pandithitta local). The mean value for control is 6.88% and for ten days after stress induction is 13.75%. For fifteen days after stress induction the proline content ranged from 5.19% (Panniyur 1) to 28.23% (Sadanandapuram local). The mean value for control is 8.53% and for fifteen days after stress induction is 17.60%.

3.1.4 Total chlorophyll (mg/g) content of black pepper genotypes

In five days after stress induction total chlorophyll content ranged from 0.502% (Thekkan) to 1.701% (Pandithitta local) (Table 4). The mean value for the control is 1.72% and for five days after stress induction is 0.94%. For ten days after stress induction the total chlorophyll content ranged from 0.313% (Padappan) to 1.261% (Annavariyan). The mean value for control is 1.46% and for ten days after stress induction is 0.67%. For fifteen days after stress induction the total chlorophyll content ranged from 0.128% (Panniyur 7) to 0.833% (Sadanandapuram local). The mean value for control is 1.28% and for fifteen days after stress induction is 0.45%.

3.1.5 Peroxidase content (activity/min/g) of black pepper genotypes

In five days after stress induction peroxidase content ranged from 1.96% (Kumbanadan) to 11.27% (Sakthi) (Table 5). The mean value for the control is 3.09% and for five days after stress induction is 6.50%. For ten days after stress induction the peroxidase content ranged from 2.13% (Panniyur 6) to 14.65% (Sadanandapuram local). The mean

value for control is 6.84% and for ten days after stress induction is 8.77%. For fifteen days after stress induction the peroxidase content ranged from 2.27% (Panniyur 6) to 18.69% (Pandithitta local). The mean value for control is 3.80% and for fifteen days after stress induction is 11.41%.

3.1.6 Polyphenol oxidase content (activity/min/g) of black pepper genotypes

In five days after stress induction polyphenol oxidase content ranged from 4.37% (Wild 2) to 8.88% (Adukkkanazhi) (Table 6). The mean value for the control is 4.02% and for five days after stress induction is 6.56%. For ten days after stress induction the polyphenol oxidase content ranged from 4.54% (Wild 2) to 11.30% (Shubhakara). The mean value for control is 4.14% and for ten days after stress induction is 7.60%. For fifteen days after stress induction the polyphenol oxidase content ranged from 6.61% (Wild 2) to 13.97% (Champakkulam local). The mean value for control is 4.28% and for fifteen days after stress induction is 9.35%.

3.1.7 Glutathione reductase content (activity/min/g) of black pepper genotypes

In five days after stress induction glutathione reductase content ranged from 2.09% (Wild 3) to 8.23% (Panniyur 7) (Table 7). The mean value for the control is 2.70% and for five days after stress induction is 4.82%. For ten days after stress induction the glutathione reductase content ranged from 2.28% (Aimpiriyan) to 11.09% (Pandithitta local). The mean value for control is 2.77% and for ten days after stress induction is 5.96%. For fifteen days after stress induction the glutathione reductase content ranged from 2.27% (Aimpiriyan) to 12.00% (Murithothan). The mean value for control is 2.92% and for fifteen days after stress induction is 7.94%.

3.1.8 Soil moisture content (%) black pepper genotypes

For five days after stress induction in control the mean value ranged between 5.41% (Kottinadan) to 20.17% (Sadanandapuram local) (Table 8). For stress induced plants the mean ranged between 3.83% (Kottinadan) to 12.88% (Sadanandapuram local). For ten days after stress induction the mean control value ranged between 7.83% (Murithothan) to 16.15% (Wild 3). The mean treatment value ranged between 3.07% (Meenadan) to 9.83% (Kuthiravali). The mean control value for 15 days after stress induction ranged between 8.12% (Cholakkodi) and 21.20% (Meenadan). Mean treated value ranged from 1.85% (Wild 3) to 6.90% (Meenadan).

Analysis of variance of soil moisture in control and different drought intensities revealed non-significant variation among different genotypes at 5 DASI, 10 DASI and control at 15th day. It suggests that there were no significant differences in soil moisture levels between the genotypes under study at those specific time points. Soil moisture was lower in the stress induced condition compared to control implying that the drought stress treatment did have a significant effect on soil moisture levels (Fig. 1).

Table 1: Mean values of relative water content (%) of 36 genotypes of black pepper

S. No	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	82.03	78.32	74.22	62.25	75.65	60.19
2	Aimpiriyan	83.90	53.47	82.51	44.60	87.18	34.64
3	Annnavariyan	83.65	58.35	83.27	52.35	86.66	37.16
4	Arikkilazhi	84.92	80.38	81.80	51.43	79.92	36.10
5	Arimulaku	81.02	68.26	78.30	60.05	79.71	48.53
6	Champakkulam Local	85.01	76.60	72.38	68.96	79.93	53.18
7	Cholakkodi	85.91	43.35	84.69	35.86	86.15	30.19
8	Karimunda	83.56	67.53	83.75	64.47	81.47	42.58
9	Karuvilanchi	79.96	70.63	82.00	60.68	87.16	56.18
10	Kottinadan	86.02	77.32	91.93	84.40	75.49	65.26
11	Kumbanadan	86.78	70.91	71.82	33.09	84.32	33.01
12	Kuthiravali	86.67	77.04	85.05	62.28	88.47	53.12
13	Malamahar	81.29	76.57	84.96	69.71	81.89	50.28
14	Meenadan	79.44	68.27	78.51	55.54	74.88	40.99
15	Murithothan	82.90	79.63	82.58	72.06	78.85	70.15
16	Naranyakodi	80.88	75.21	88.11	64.26	84.07	46.14
17	Nithyakalyani	87.80	86.73	83.20	80.24	80.25	30.80
18	Padappan	84.09	67.86	83.95	50.24	82.15	41.28
19	Palakkodi	83.69	74.32	77.08	69.35	83.10	37.69
20	Pandithitta Local	84.36	76.30	81.02	75.41	80.29	71.83
21	Panniyur 1	78.24	71.22	80.87	61.73	75.31	59.43
22	Panniyur 2	81.27	60.16	88.44	58.37	78.67	47.31
23	Panniyur 5	79.28	60.52	71.28	50.96	72.04	35.10
24	Panniyur 6	86.38	65.03	86.17	53.97	85.02	42.79
25	Panniyur 7	81.88	59.81	86.20	47.44	83.92	26.66
26	Panniyur 8	82.18	69.72	81.77	60.26	80.83	34.19
27	Perumkodi	80.62	64.22	76.42	48.29	77.43	25.65
28	Sadanandapuram Local	92.58	85.74	88.93	85.04	81.27	68.37
29	Sakthi	81.00	63.27	81.85	57.71	83.06	39.61
30	Subhakara	83.61	72.10	86.84	61.22	80.55	52.92
31	Thekkan	82.87	77.20	82.79	48.97	84.14	41.03
32	Vellamundi	82.99	65.48	87.22	59.07	81.67	46.35
33	Velliyarmunda	85.87	71.82	84.86	65.84	58.46	46.01
34	Wild 1	80.14	79.66	75.90	72.25	83.29	38.07
35	Wild 2	78.66	76.80	77.64	75.29	78.09	62.47
36	Wild 3	81.68	79.59	76.71	78.54	72.47	56.76

Table 2: Mean values of membrane leakage (%) of 36 genotypes of black pepper

S. No	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	24.81	50.02	31.05	31.54	29.66	53.67
2	Aimpiriyan	21.76	19.97	22.73	41.94	25.87	86.42
3	Annnavariyan	40.52	57.56	43.70	66.62	45.00	77.40
4	Arikkilazhi	12.18	20.13	21.21	42.96	23.84	66.78
5	Arimulaku	32.79	48.47	37.18	55.28	35.17	84.27
6	Champakkulam Local	27.56	54.35	26.61	64.39	26.09	80.72
7	Cholakkodi	32.53	51.33	42.01	88.23	40.61	84.59
8	Karimunda	43.47	51.17	39.75	61.94	47.37	54.17
9	Karuvilanchi	54.66	60.70	55.93	74.70	57.15	78.55
10	Kottinadan	32.14	34.52	33.54	42.32	38.25	55.11
11	Kumbanadan	34.72	59.65	38.25	74.83	35.16	88.09
12	Kuthiravali	31.44	55.08	34.39	70.42	36.32	83.39
13	Malamahar	35.29	62.37	32.09	72.20	38.00	83.94
14	Meenadan	48.79	64.48	43.89	73.84	41.31	83.74
15	Murithothan	30.56	62.37	33.89	74.19	43.58	80.50
16	Naranyakodi	43.42	53.03	44.18	76.22	42.56	83.94
17	Nithyakalyani	15.42	32.72	15.15	52.74	18.20	71.82
18	Padappan	41.06	66.48	42.30	73.86	45.01	80.66
19	Palakkodi	17.03	24.39	13.80	38.33	14.83	58.30
20	Pandithitta Local	25.95	30.96	28.21	46.12	29.85	52.64
21	Panniyur 1	20.74	36.49	22.10	40.52	28.28	55.71
22	Panniyur 2	28.19	43.27	34.60	51.82	35.73	66.41
23	Panniyur 5	27.04	35.84	29.90	49.87	28.17	61.54
24	Panniyur 6	35.22	48.38	39.14	56.36	37.30	72.23
25	Panniyur 7	36.43	45.08	35.44	56.19	37.38	72.51
26	Panniyur 8	44.94	64.86	45.63	70.61	46.15	72.07
27	Perumkodi	37.50	45.76	41.62	62.51	37.26	59.12
28	Sadanandapuram Local	14.16	21.63	15.08	24.52	18.61	27.32
29	Sakthi	23.82	53.30	28.94	71.00	27.83	78.95
30	Subhakara	22.04	61.10	24.65	73.43	30.75	79.64
31	Thekkan	22.83	40.40	31.26	62.05	31.88	79.62
32	Vellamundi	28.20	43.12	35.39	65.48	36.67	77.80
33	Velliyarmunda	13.32	38.06	21.40	68.93	22.23	84.69
34	Wild 1	27.65	31.53	26.28	41.03	24.52	65.11
35	Wild 2	68.45	72.48	62.36	75.99	68.16	73.67
36	Wild 3	34.70	57.38	39.33	67.15	35.83	77.67

Table 3: Mean values of proline content (μ mol/g) of 36 genotypes of black pepper

S. No	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	6.21	17.35	10.37	20.08	9.94	16.68
2	Aimpiriyan	2.61	6.26	4.41	6.34	5.55	9.37
3	Annavariyan	5.77	11.72	8.49	12.16	7.89	20.57
4	Arikkilazhi	4.99	8.04	7.62	10.55	5.79	17.11
5	Arimulaku	8.93	14.59	11.34	16.26	11.50	26.38
6	Champakkulam Local	1.51	4.53	4.81	7.07	3.22	8.07
7	Cholakkodi	1.75	4.24	3.46	5.51	2.69	18.92
8	Karimunda	5.31	13.95	7.82	17.46	13.37	20.36
9	Karuvilanchi	3.93	14.77	9.77	20.90	10.41	21.76
10	Kottinadan	7.63	10.80	9.95	17.31	10.09	25.47
11	Kumbanadan	5.04	10.03	5.22	9.40	7.32	8.51
12	Kuthiravali	6.28	11.67	7.24	13.10	9.73	14.22
13	Malamahar	5.52	12.56	8.56	14.97	4.45	12.98
14	Meenadan	6.95	13.88	10.42	17.37	10.31	15.07
15	Murithothan	4.63	6.42	5.36	11.04	7.17	16.67
16	Naranyakodi	7.99	12.59	10.80	15.02	8.27	17.72
17	Nithyakalyani	5.38	16.62	6.09	17.21	6.91	16.07
18	Padappan	8.29	14.61	9.37	16.89	10.50	19.93
19	Palakkodi	4.30	16.67	4.50	26.01	7.47	22.54
20	Pandithitta Local	3.51	17.95	8.00	24.57	8.13	27.48
21	Panniyur 1	4.06	5.19	3.62	3.94	3.61	4.89
22	Panniyur 2	8.55	10.26	7.75	10.80	8.05	15.03
23	Panniyur 5	6.96	13.46	9.94	14.39	9.48	15.16
24	Panniyur 6	4.55	7.20	8.78	13.19	14.89	18.45
25	Panniyur 7	8.98	10.38	10.72	13.56	9.24	15.01
26	Panniyur 8	6.34	12.43	10.18	13.22	9.63	15.31
27	Perumkodi	4.71	10.34	4.94	11.07	7.34	21.11
28	Sadanandapuram Local	5.16	16.47	7.57	19.89	11.67	28.23
29	Sakthi	4.73	12.26	4.77	21.43	8.55	21.63
30	Subhakara	9.88	11.69	10.39	18.37	10.33	19.08
31	Thekkan	8.42	11.58	9.81	18.73	6.72	12.46
32	Vellamundi	5.13	9.38	8.60	13.99	7.03	20.12
33	Velliyarmunda	3.43	11.54	4.42	15.72	6.82	15.23
34	Wild 1	2.60	6.35	3.39	7.82	3.41	9.21
35	Wild 2	3.09	4.96	4.56	5.50	4.08	6.04
36	Wild 3	4.02	4.28	4.05	6.27	5.34	11.90

Table 4: Mean values of total chlorophyll (mg/g) content of 36 genotypes of black pepper

S. No	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	1.271	0.579	1.505	0.418	0.750	0.450
2	Aimpiriyan	1.490	0.648	1.601	0.563	1.475	0.595
3	Annavariyan	2.532	1.648	2.774	1.261	1.535	0.405
4	Arikkilazhi	1.259	0.980	1.504	0.745	1.005	0.281
5	Arimulaku	2.074	1.573	2.026	0.560	1.971	0.344
6	Champakkulam Local	0.888	0.752	0.975	0.443	0.827	0.358
7	Cholakkodi	1.121	0.620	1.574	0.401	1.415	0.458
8	Karimunda	2.594	1.162	1.698	0.442	1.668	0.311
9	Karuvilanchi	1.119	0.972	1.066	0.614	1.069	0.536
10	Kottinadan	2.079	0.707	2.459	0.416	1.740	0.361
11	Kumbanadan	1.259	0.772	1.659	0.805	1.664	0.675
12	Kuthiravali	1.966	1.070	1.454	0.622	1.698	0.383
13	Malamahar	2.678	0.940	2.072	0.572	2.100	0.321
14	Meenadan	1.069	0.772	1.093	0.536	1.006	0.492
15	Murithothan	1.825	1.231	1.725	1.151	1.620	0.790
16	Naranyakodi	2.636	0.915	1.913	0.607	2.032	0.384
17	Nithyakalyani	1.402	1.135	1.182	0.900	1.043	0.800
18	Padappan	0.965	0.680	0.920	0.313	0.853	0.268
19	Palakkodi	1.731	1.308	1.460	1.135	1.265	0.387
20	Pandithitta Local	1.756	1.701	1.336	1.125	1.610	0.806
21	Panniyur 1	0.990	0.930	0.935	0.862	0.749	0.729
22	Panniyur 2	1.582	0.868	1.590	0.450	1.488	0.377
23	Panniyur 5	0.964	0.836	0.729	0.703	0.725	0.540
24	Panniyur 6	1.537	0.876	1.295	0.833	1.296	0.344
25	Panniyur 7	1.517	0.887	1.190	0.591	1.841	0.128
26	Panniyur 8	1.278	0.646	2.126	0.460	1.367	0.398
27	Perumkodi	1.145	0.830	0.975	0.703	0.854	0.803
28	Sadanandapuram Local	1.399	0.948	1.001	0.836	1.615	0.833
29	Sakthi	1.536	0.846	1.992	0.559	1.581	0.385
30	Subhakara	1.559	0.691	0.865	0.655	0.855	0.560
31	Thekkan	1.054	0.502	1.877	0.327	1.979	0.362
32	Vellamundi	2.260	0.987	1.873	0.732	1.795	0.491
33	Velliyarmunda	1.977	0.789	1.762	0.497	1.875	0.460
34	Wild 1	1.271	0.802	1.911	0.880	1.566	0.480
35	Wild 2	1.246	1.045	1.388	0.962	1.325	0.281
36	Wild 3	1.773	1.210	1.301	0.418	1.040	0.151

Table 5: Mean values of peroxidase content (activity/min/g) of 36 genotypes of black pepper

	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	4.62	11.26	3.81	12.50	2.67	13.36
2	Aimpiriyan	3.12	6.13	8.87	14.35	4.06	5.37
3	Annavariyan	3.78	4.88	3.52	7.08	2.96	5.98
4	Arikkilazhi	2.25	6.19	2.10	8.13	2.43	17.32
5	Arimulaku	4.18	6.97	4.58	11.41	4.29	15.50
6	Champakkulam Local	2.15	6.92	1.89	7.00	3.20	8.28
7	Cholakkodi	0.21	4.45	1.83	5.38	1.16	5.78
8	Karimunda	2.38	4.42	2.95	4.55	2.80	4.73
9	Karuvilanchi	2.28	8.08	4.01	9.66	4.74	11.07
10	Kottinadan	4.54	8.76	6.70	13.71	3.83	16.58
11	Kumbanadan	0.83	1.96	2.79	3.33	2.32	5.31
12	Kuthiravali	3.64	5.69	2.48	6.88	4.85	11.52
13	Malamahar	5.30	9.97	5.47	11.09	5.49	12.19
14	Meenadan	4.86	7.50	4.07	8.45	4.78	13.01
15	Murithothan	3.48	7.16	4.50	8.01	5.96	11.59
16	Narayakodi	5.44	7.35	3.08	10.12	4.57	12.43
17	Nithyakalyani	0.73	3.01	1.69	7.20	2.00	8.21
18	Padappan	3.23	7.89	4.86	9.73	5.08	15.45
19	Palakkodi	3.43	5.43	3.03	6.68	3.31	11.66
20	Pandithitta Local	2.74	8.03	2.08	15.74	2.75	18.69
21	Panniyur 1	2.43	5.92	1.11	6.41	1.72	6.86
22	Panniyur 2	3.52	5.51	5.33	11.92	4.23	12.30
23	Panniyur 5	2.25	5.44	1.81	9.14	1.83	13.43
24	Panniyur 6	1.43	1.99	1.26	2.13	1.25	2.27
25	Panniyur 7	4.29	7.99	3.07	10.02	3.58	12.19
26	Panniyur 8	3.15	4.60	3.82	5.38	3.42	8.34
27	Perumkodi	5.25	7.95	4.77	8.76	7.00	10.85
28	Sadanandapuram Local	4.35	10.94	4.68	14.65	4.16	16.41
29	Sakthi	6.22	11.27	4.49	12.22	6.08	13.08
30	Subhakara	2.13	6.80	1.80	11.29	3.43	13.68
31	Thekkan	2.27	4.46	1.71	4.69	2.80	5.57
32	Vellamundi	2.35	4.01	3.53	6.23	4.16	9.26
33	Velliyarmunda	3.88	8.36	4.71	10.21	4.62	10.57
34	Wild 1	1.93	6.83	3.35	8.39	3.62	14.75
35	Wild 2	1.55	3.19	1.58	3.54	1.16	4.46
36	Wild 3	4.08	7.03	4.76	9.89	4.82	13.06

Table 6: Mean values of polyphenol oxidase content (activity/min/g) of 36 genotypes of black pepper

	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	5.39	8.88	2.93	9.56	5.29	10.07
2	Aimpiriyan	4.80	5.87	3.82	6.66	3.87	8.07
3	Annavariyan	4.60	6.02	4.47	9.27	4.77	9.54
4	Arikkilazhi	4.44	7.85	4.24	8.01	4.87	7.93
5	Arimulaku	4.56	5.88	3.99	6.29	3.77	8.10
6	Champakkulam Local	4.56	7.28	3.00	10.10	5.80	13.97
7	Cholakkodi	5.22	6.58	5.76	8.40	4.47	9.98
8	Karimunda	4.71	6.69	4.99	7.59	3.64	8.34
9	Karuvilanchi	5.48	6.80	4.82	7.11	5.10	8.60
10	Kottinadan	3.26	7.81	4.57	8.62	3.19	11.16
11	Kumbanadan	1.14	4.86	3.38	5.31	2.81	10.00
12	Kuthiravali	3.67	5.74	3.71	7.20	4.13	9.99
13	Malamahar	2.80	6.20	3.26	7.01	3.93	8.43
14	Meenadan	4.55	6.54	4.31	7.26	5.27	10.26
15	Murithothan	4.08	7.46	4.34	8.10	4.84	11.45
16	Narayakodi	3.98	7.64	2.71	8.28	2.93	9.08
17	Nithyakalyani	0.73	5.53	1.83	6.26	1.91	9.61
18	Padappan	4.63	4.79	3.19	5.91	4.91	7.27
19	Palakkodi	3.60	6.73	3.37	8.99	4.84	9.67
20	Pandithitta Local	6.13	7.50	4.79	6.41	6.65	10.73
21	Panniyur 1	4.82	7.99	5.57	8.36	4.48	9.47
22	Panniyur 2	4.56	5.92	5.19	5.84	4.80	8.99
23	Panniyur 5	3.49	5.43	3.45	6.56	3.32	8.29
24	Panniyur 6	4.67	6.39	4.46	7.16	4.58	8.93
25	Panniyur 7	3.58	5.86	3.86	8.63	4.27	10.17
26	Panniyur 8	4.44	6.85	4.41	7.12	4.26	7.27
27	Perumkodi	4.73	6.34	2.79	6.79	4.76	7.95
28	Sadanandapuram Local	3.18	8.36	5.30	8.45	6.08	9.88
29	Sakthi	5.04	5.52	3.93	6.21	4.39	7.03
30	Subhakara	4.92	8.27	4.12	11.30	4.83	12.00
31	Thekkan	5.43	7.99	6.51	7.14	6.08	8.31
32	Vellamundi	8.98	6.50	0.36	0.10	4.41	11.89
33	Velliyarmunda	4.47	5.03	4.29	8.31	4.69	7.49
34	Wild 1	1.95	6.43	3.01	7.82	3.83	8.76
35	Wild 2	2.43	4.37	2.12	4.54	3.87	6.61
36	Wild 3	2.62	6.44	2.64	9.23	2.25	10.37

Table 7: Mean values of glutathione reductase content (activity/min/g) of 36 genotypes of black pepper

	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	3.96	7.01	3.86	7.90	3.49	9.22
2	Aimpiriyan	2.60	2.77	2.05	2.28	1.35	2.27
3	Annavariyan	2.12	6.19	1.50	8.02	1.62	9.34
4	Arikkilazhi	3.43	5.62	4.06	4.46	3.57	4.61
5	Arimulaku	1.07	4.57	2.29	4.81	1.70	5.47
6	Champakkulam Local	1.55	2.95	2.26	6.61	1.45	4.28
7	Cholakkodi	3.70	4.02	2.99	5.22	3.57	6.31
8	Karimunda	2.62	4.13	2.22	4.24	2.56	8.63
9	Karuvilanchi	1.94	3.77	1.39	4.72	1.18	8.59
10	Kottinadan	3.93	5.93	3.54	6.84	4.03	10.24
11	Kumbanadan	0.66	2.99	1.75	9.61	1.78	4.06
12	Kuthiravali	2.13	4.91	2.52	5.07	2.19	8.83
13	Malamahar	3.66	6.15	4.16	7.64	3.42	8.13
14	Meenadan	1.21	2.67	1.62	3.64	1.40	4.80
15	Murithothan	4.66	5.68	4.54	6.75	4.37	12.00
16	Narayakodi	4.69	6.91	4.93	6.49	4.88	7.78
17	Nithyakalyani	1.77	4.22	1.45	5.85	1.67	8.89
18	Padappan	3.77	5.44	3.85	6.45	4.40	6.89
19	Palakkodi	2.03	6.99	1.17	7.30	2.68	9.38
20	Pandithitta Local	3.51	6.07	2.61	11.09	2.49	11.80
21	Panniyur 1	3.58	4.02	2.37	4.99	3.26	8.02
22	Panniyur 2	3.75	5.15	3.95	6.84	4.36	9.20
23	Panniyur 5	5.61	6.91	5.71	8.58	4.74	9.75
24	Panniyur 6	0.76	2.35	1.57	3.18	1.95	3.62
25	Panniyur 7	3.08	8.23	2.95	9.53	2.69	11.96
26	Panniyur 8	2.80	4.76	2.87	7.74	1.95	8.58
27	Perumkodi	3.43	5.18	3.01	6.18	4.91	8.71
28	Sadanandapuram Local	3.67	7.78	3.77	8.04	3.47	10.18
29	Sakthi	3.75	5.94	2.97	6.24	3.48	7.83
30	Subhakara	1.29	2.01	1.23	2.63	2.64	8.21
31	Thekkan	3.12	6.27	3.38	7.00	3.75	7.82
32	Vellamundi	3.18	6.21	2.53	6.79	3.39	8.57
33	Velliyarmunda	2.11	4.68	1.88	5.79	2.66	8.82
34	Wild 1	0.27	2.26	1.15	2.72	1.96	3.96
35	Wild 2	1.50	3.28	1.77	5.51	1.33	8.24
36	Wild 3	0.76	2.09	1.62	3.45	1.69	3.06

Table 8: Mean values of Soil moisture content (%) for 36 genotypes of black pepper

	Genotypes	5 DASI		10 DASI		15 DASI	
		Control	SI	Control	SI	Control	SI
1	Adukkanaazhi	15.34	9.98	11.40	3.75	14.15	2.75
2	Aimpiriyan	10.11	7.05	13.70	3.31	10.38	2.26
3	Annavariyan	19.22	10.88	15.77	8.15	17.94	2.70
4	Arikkilazhi	8.32	6.45	13.40	4.41	12.68	3.55
5	Arimulaku	14.19	8.44	15.95	6.79	13.35	5.64
6	Champakkulam Local	6.67	5.20	11.15	6.05	11.65	2.45
7	Cholakkodi	13.07	7.92	9.65	3.55	8.12	2.73
8	Karimunda	12.91	6.37	11.99	7.50	13.77	5.02
9	Karuvilanchi	13.40	9.45	14.24	5.82	17.46	4.16
10	Kottinadan	5.41	3.83	12.10	5.25	9.60	2.55
11	Kumbanadan	9.83	8.91	12.75	5.85	9.29	2.27
12	Kuthiravali	14.12	10.08	12.83	9.83	11.79	1.94
13	Malamahar	13.84	10.22	12.46	9.69	15.64	4.89
14	Meenadan	13.64	7.67	14.60	3.07	21.20	6.90
15	Murithothan	19.35	6.03	7.89	4.18	9.69	5.92
16	Narayakodi	13.55	9.00	14.95	4.90	15.20	2.70
17	Nithyakalyani	10.62	7.30	15.16	5.65	10.62	2.37
18	Padappan	12.53	7.18	14.98	4.77	12.71	3.70
19	Palakkodi	11.72	9.25	12.10	8.00	10.96	2.84
20	Pandithitta Local	9.51	6.20	12.70	3.70	11.65	2.80
21	Panniyur 1	8.20	6.49	9.14	5.05	10.75	3.00
22	Panniyur 2	11.41	8.34	10.01	6.62	10.82	2.73
23	Panniyur 5	11.06	8.28	12.65	5.65	11.15	1.98
24	Panniyur 6	10.57	6.16	12.98	4.03	10.69	2.37
25	Panniyur 7	13.97	8.32	13.93	6.39	13.69	2.40
26	Panniyur 8	15.71	9.41	13.68	5.30	11.90	2.89
27	Perumkodi	12.21	8.17	11.78	5.60	14.82	2.51
28	Sadanandapuram Local	20.17	12.88	14.15	6.55	14.65	2.20
29	Sakthi	11.03	9.11	10.11	6.94	12.49	2.05
30	Subhakara	10.36	9.69	12.78	7.26	12.52	6.43
31	Thekkan	13.31	9.83	10.32	6.58	12.10	4.34
32	Vellamundi	12.56	8.48	12.19	5.78	13.38	3.05
33	Velliyarmunda	11.15	7.98	11.36	5.82	11.82	4.15
34	Wild 1	9.14	7.59	11.10	4.90	14.56	3.14
35	Wild 2	11.45	9.40	13.45	6.08	13.10	2.15
36	Wild 3	12.64	10.60	16.15	7.80	11.60	1.85
	SE(m)	1.607	1.808	1.389	1.858	2.059	0.779
	CD	4.61	NS	3.985	NS	NS	2.235

3.2 Identification of resistant genotypes in the germplasm

The results recorded were interpreted in two-way factorial analysis using GRAPES software version 1.1.0. (Gopinath *et al.*, 2021) ^[9] to identify resistant genotypes by statistical analysis of data. Analysis of variance for various physiological and biochemical characters are presented in Table 9-15.

3.2.1 Relative Water Content (RWC) (%)

The relative water content differed significantly among black pepper genotypes and for different stress intensities. Maximum RWC was observed for Sadanandapuram local (80.104%) followed by Pandithitta local (75.95%), Kottinadan (75.61%) and Murithothan (75.17%). Lowest RWC was observed for Cholakkodi (48.88%) and Perumkodi (53.89%).

3.2.2 Membrane Leakage (%)

Significant variation was observed for all the genotypes and among different days after stress induction. Lowest ML was recorded by Sadanandapuram local (23.02), which was on par with Palakkodi (33.95), Arikilazhi (38.43), Pandithitta local (39.89) and Panniyur 1(40.24). Highest ML was recorded by Wild 2 (72.57), Karuvilanchi (67.77) and Padappan (66.50).

Table 9: Effect of drought on relative water content (%) of different black pepper genotypes

Genotype	RWC (%)				
	Control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	75.65	78.32	62.25	60.19	69.10
Aimpiriyan	87.18	53.47	44.60	34.64	54.97
Annavariyan	86.66	58.35	52.35	37.16	58.63
Arikkilazhi	79.92	80.38	51.43	36.10	61.95
Arimulaku	79.71	68.26	60.05	48.53	64.14
Champakkulam local	79.93	76.60	68.96	53.18	69.67
Cholakkodi	86.15	43.35	35.86	30.19	48.89
Karimunda	81.47	67.53	64.47	42.58	64.01
Karuvilanchi	87.16	70.63	60.68	56.18	68.66
Kottinadan	75.49	77.32	84.40	65.26	75.62
Kumbanadan	84.32	70.91	33.09	33.01	55.33
Kuthiravali	88.47	77.04	62.28	53.12	70.23
Malamahar	81.89	76.57	69.71	50.28	69.61
Meenadan	74.88	68.27	55.54	40.99	59.92
Murithothan	78.85	79.63	72.06	70.15	75.17
Narayakodi	84.07	75.21	64.26	46.14	67.42
Nithyakalyani	80.25	86.73	80.24	30.80	69.50
Padappan	82.15	67.86	50.24	41.28	60.38
Palakkodi	83.10	74.32	69.35	37.69	66.12
Pandithitta local	80.29	76.30	75.41	71.83	75.96
Panniyur 1	75.31	71.22	61.73	59.43	66.92
Panniyur 2	78.67	60.16	58.37	47.31	61.13
Panniyur 5	72.04	60.52	50.96	35.10	54.66
Panniyur 6	85.02	65.03	53.97	42.79	61.70
Panniyur 7	83.92	59.81	47.44	26.66	54.46
Panniyur 8	80.83	69.72	60.26	34.19	61.25
Perumkodi	77.43	64.22	48.29	25.65	53.89
Sadanandapuram local	81.27	85.74	85.04	68.37	80.10
Sakthi	83.06	63.27	57.71	39.61	60.91
Subhakara	80.55	72.10	61.22	52.92	66.70
Thekkan	84.14	77.20	48.97	41.03	62.83
Vellamundi	81.67	65.48	59.07	46.35	63.14
Velliyarmunda	58.46	71.82	65.84	46.01	60.53
Wild 1	83.29	79.66	72.25	38.07	68.32
Wild 2	78.09	76.80	75.29	62.47	73.16
Wild 3	72.47	79.59	78.54	56.76	71.84
Mean	80.38	70.82	61.17	46.17	
	SE(m)	CD			
Genotypes	3.432	9.592			
DASI	1.144	3.197			

Table 10: Effect of drought on membrane leakage (%) of different black pepper genotypes

Genotype	Membrane Leakage (%)				
	Control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	29.66	50.02	31.54	53.67	41.22
Aimpiriyan	25.87	19.97	41.94	86.42	43.55
Annavariyan	45.00	57.56	66.62	77.40	61.64
Arikkilazhi	23.84	20.13	42.96	66.78	38.43
Arimulaku	35.17	48.47	55.28	84.27	55.80
Champakkulam local	26.09	54.35	64.39	80.72	56.39
Cholakkodi	40.61	51.33	88.23	84.59	66.19
Karimunda	47.37	51.17	61.94	54.17	53.66
Karuvilanchi	57.15	60.70	74.70	78.55	67.77
Kottinadan	38.25	34.52	42.32	55.11	42.55
Kumbanadan	35.16	59.65	74.83	88.09	64.43
Kuthiravali	36.32	55.08	70.42	83.39	61.30
Malamahar	38.00	62.37	72.20	83.94	64.13
Meenadan	41.31	64.48	73.84	83.74	65.84
Murithothan	43.58	62.37	74.19	80.50	65.16
Narayakodi	42.56	53.03	76.22	83.94	63.94
Nithyakalyani	18.20	32.72	52.74	71.82	43.87
Padappan	45.01	66.48	73.86	80.66	66.50
Palakkodi	14.83	24.39	38.33	58.30	33.96
Pandithitta local	29.85	30.96	46.12	52.64	39.89
Panniyur 1	28.28	36.49	40.52	55.71	40.25
Panniyur 2	35.73	43.27	51.82	66.41	49.31
Panniyur 5	28.17	35.84	49.87	61.54	43.85
Panniyur 6	37.30	48.38	56.36	72.23	53.57
Panniyur 7	37.38	45.08	56.19	72.51	52.79
Panniyur 8	46.15	64.86	70.61	72.07	63.42
Perumkodi	37.26	45.76	62.51	59.12	51.16
Sadanandapuram local	18.61	21.63	24.52	27.32	23.02
Sakthi	27.83	53.30	71.00	78.95	57.77
Subhakara	30.75	61.10	73.43	79.64	61.23
Thekkan	31.88	40.40	62.05	79.62	53.49
Vellamundi	36.67	43.12	65.48	77.80	55.77
Velliyarmunda	22.23	38.06	68.93	84.69	53.48
Wild 1	24.52	31.53	41.03	65.11	40.55
Wild 2	68.16	72.48	75.99	73.67	72.57
Wild 3	35.83	57.38	67.15	77.67	59.51
Mean	35.02	47.18	60.003	72.02	
	SE (m)	CD			
Genotypes	4.833	13.509			
DASI	1.611	4.503			

3.2.3 Proline content (μ mol/g)

Highest content of proline was recorded by Pandithitta local (19.53) which was on par with Sadanandapuram local (19.06), Palakkodi (18.17), Arimulaku (17.18) and Karuvilanchi (16.95). Significant variation was observed for the character among genotypes and different stress intensities. Mean proline concentration was highest in plants after 15 days (16.79) of withholding irrigation followed by 10 days (14.09) and 5 days (11.03) respectively. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration is usually higher in stress tolerant than in stress sensitive plants (Silvente *et al.*, 2012) ^[16].

3.2.4 Chlorophyll content (mg/g)

Black pepper genotypes varied significantly for chlorophyll content. Highest chlorophyll content was shown by Pandithitta local (1.31) followed by Annavariyan (1.21), Murithothan (1.19), Arimulaku (1.11) and Sadanandapuram local (1.058). Significant variation was observed among different periods of drought in which control plants had highest chlorophyll content followed by plants stressed for 5 days, 10 days and 15 days respectively.

Table 11: Effect of drought on proline content (μ mol/g) of different black pepper genotypes

Genotype	Proline content (μ mol/g)				
	Control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	9.94	17.35	20.08	16.68	16.01
Aimpiriyan	5.55	6.26	6.34	9.37	6.88
Annavariyan	7.89	11.72	12.16	20.57	13.08
Arikkilazhi	5.79	8.04	10.55	17.11	10.37
Arimulaku	11.50	14.59	16.26	26.38	17.18
Champakkulam local	3.22	4.53	7.07	8.07	5.72
Cholakkodi	2.69	4.24	5.51	18.92	7.84
Karimunda	13.37	13.95	17.46	20.36	16.29
Karuvilanchi	10.41	14.77	20.90	21.76	16.96
Kottinadan	10.09	10.80	17.31	25.47	15.92
Kumbanadan	7.32	10.03	9.40	8.51	8.81
Kuthiravali	9.73	11.67	13.10	14.22	12.18
Malamahar	4.45	12.56	14.97	12.98	11.24
Meenadan	10.31	13.88	17.37	15.07	14.16
Murithothan	7.17	6.42	11.04	16.67	10.32
Narayakodi	8.27	12.59	15.02	17.72	13.40
Nithyakalyani	6.91	16.62	17.21	16.07	14.20
Padappan	10.50	14.61	16.89	19.93	15.48
Palakkodi	7.47	16.67	26.01	22.54	18.17
Pandithitta local	8.13	17.95	24.57	27.48	19.53
Panniyur 1	3.61	5.19	3.94	4.89	4.40
Panniyur 2	8.05	10.26	10.80	15.03	11.03
Panniyur 5	9.48	13.46	14.39	15.16	13.12
Panniyur 6	14.89	7.20	13.19	18.45	13.43
Panniyur 7	9.24	10.38	13.56	15.01	12.05
Panniyur 8	9.63	12.43	13.22	15.31	12.65
Perumkodi	7.34	10.34	11.07	21.11	12.46
Sadanandapuram local	11.67	16.47	19.89	28.23	19.06
Sakthi	8.55	12.26	21.43	21.63	15.97
Subhakara	10.33	11.69	18.37	19.08	14.87
Thekkan	6.72	11.58	18.73	12.46	12.37
Vellamundi	7.03	9.38	13.99	20.12	12.63
Velliyarmunda	6.82	11.54	15.72	15.23	12.33
Wild 1	3.41	6.35	7.82	9.21	6.70
Wild 2	4.08	4.96	5.50	6.04	5.14
Wild 3	5.34	4.28	6.27	11.90	6.95
Mean	7.97	11.03	14.09	16.79	
	SE (m)	CD			
Genotypes	1.504	4.230			
DASI	0.501	1.401			

Table 12: Effect of drought on chlorophyll content (mg/g) of different black pepper genotypes

Genotype	Chlorophyll Content (mg/g)				
	Control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	0.750	0.579	0.418	0.450	0.549
Aimpiriyan	1.475	0.648	0.563	0.595	0.820
Annavariyan	1.535	1.648	1.261	0.405	1.213
Arikkilazhi	1.005	0.980	0.745	0.281	0.753
Arimulaku	1.971	1.573	0.560	0.344	1.112
Champakkulam local	0.827	0.752	0.443	0.358	0.595
Cholakkodi	1.415	0.620	0.401	0.458	0.724
Karimunda	1.668	1.162	0.442	0.311	0.896
Karuvilanchi	1.069	0.972	0.614	0.536	0.798
Kottinadan	1.740	0.707	0.416	0.361	0.806
Kumbanadan	1.664	0.772	0.805	0.675	0.979
Kuthiravali	1.698	1.070	0.622	0.383	0.944
Malamahar	2.100	0.940	0.572	0.321	0.983
Meenadan	1.006	0.772	0.536	0.492	0.701
Murithothan	1.620	1.231	1.151	0.790	1.198
Narayakodi	2.032	0.915	0.607	0.384	0.984
Nithyakalyani	1.043	1.135	0.900	0.800	0.969
Padappan	0.853	0.680	0.313	0.268	0.528
Palakkodi	1.265	1.308	1.135	0.387	1.024
Pandithitta local	1.610	1.701	1.125	0.806	1.310
Panniyur 1	0.749	0.930	0.862	0.729	0.817
Panniyur 2	1.488	0.868	0.450	0.377	0.796
Panniyur 5	0.725	0.836	0.703	0.540	0.701
Panniyur 6	1.296	0.876	0.833	0.344	0.837
Panniyur 7	1.841	0.887	0.591	0.128	0.862
Panniyur 8	1.367	0.646	0.460	0.398	0.718
Perumkodi	0.854	0.830	0.703	0.803	0.797
Sadanandapuram local	1.615	0.948	0.836	0.833	1.058
Sakthi	1.581	0.846	0.559	0.385	0.843
Subhakara	0.855	0.691	0.655	0.560	0.690
Thekkan	1.979	0.502	0.327	0.362	0.793
Vellamundi	1.795	0.987	0.732	0.491	1.001
Velliyarmunda	1.875	0.789	0.497	0.460	0.905
Wild 1	1.566	0.802	0.880	0.480	0.932
Wild 2	1.325	1.045	0.962	0.281	0.903
Wild 3	1.040	1.210	0.418	0.151	0.705
Mean	1.397	0.940	0.669	0.465	
	SE (m)	CD			
Genotypes	0.126	0.353			
DASI	0.042	0.118			

3.2.5 Peroxidase content (activity/min/g)

Variation among the 36 genotypes and different stress intensities were significant. Peroxidase content was highest for Sadanandapuram local (11.54) followed by Pandithitta local (11.3), Kottinadan (10.72), and Sakthi (10.66). Lowest mean peroxidase content was recorded for Panniyur 6 (1.91) followed by Wild 2 (3.09) and Kumbhanadan (3.23). Peroxidase content was highest at 15 DASI (10.86) followed by 10 DASI and 5 DASI respectively.

3.2.6 Polyphenol oxidase (activity/min/g)

Significant variation for the character was observed among genotypes and for different stress intensities. Highest mean polyphenol oxidase content was recorded for Champakkulam local (9.28) followed by Shubakara (9.09), adukanazhi (8.45) and Sadanandapuram local (8.19). Lowest mean was recorded by Wild 2 (4.84) followed by Padappan (5.72) and Vellamundi (5.73). Highest mean content was observed under severe stress ie., 15 DASI followed by 10 DASI, 5 DASI and control.

Table 13: Effect of drought on peroxidase content (activity/min/g) of different black pepper genotypes

Genotype	Peroxidase Content (activity/min/g)				
	control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	2.67	11.26	12.5	13.36	9.95
Aimpiriyan	4.06	6.13	14.35	5.37	7.48
Annavariyan	2.96	4.88	7.08	5.98	5.22
Arikkilazhi	2.43	6.19	8.13	17.32	8.52
Arimulaku	4.29	6.97	11.41	15.50	9.54
Champakkulam local	3.20	6.92	7.00	8.28	6.35
Cholakkodi	1.16	4.45	5.38	5.78	4.19
Karimunda	2.80	4.42	4.55	4.73	4.12
Karuvilanchi	4.74	8.08	9.66	11.07	8.39
Kottinadan	3.83	8.76	13.71	16.58	10.72
Kumbanadan	2.32	1.96	3.33	5.31	3.23
Kuthiravali	4.85	5.69	6.88	11.52	7.24
Malamahar	5.49	9.97	11.09	12.19	9.69
Meenadan	4.78	7.50	8.45	13.01	8.43
Murithothan	5.96	7.16	8.01	11.59	8.18
Narayakodi	4.57	7.35	10.12	12.43	8.62
Nithyakalyani	2.00	3.01	7.20	8.21	5.10
Padappan	5.08	7.89	9.73	15.45	9.54
Palakkodi	3.31	5.43	6.68	11.66	6.77
Pandithitta local	2.75	8.03	15.74	18.69	11.30
Panniyur 1	1.72	5.92	6.41	6.86	5.23
Panniyur 2	4.23	5.51	11.92	12.30	8.49
Panniyur 5	1.83	5.44	9.14	13.43	7.46
Panniyur 6	1.25	1.99	2.13	2.27	1.91
Panniyur 7	3.58	7.99	10.02	12.19	8.45
Panniyur 8	3.42	4.60	5.38	8.34	5.43
Perumkodi	7.00	7.95	8.76	10.85	8.64
Sadanandapuram local	4.16	10.94	14.65	16.41	11.54
Sakthi	6.08	11.27	12.22	13.08	10.66
Subhakara	3.43	6.80	11.29	13.68	8.80
Thekkan	2.80	4.46	4.69	5.57	4.38
Vellamundi	4.16	4.01	6.23	9.26	5.92
Velliyarmunda	4.62	8.36	10.21	10.57	8.44
Wild 1	3.62	6.83	8.39	14.75	8.40
Wild 2	1.16	3.19	3.54	4.46	3.09
Wild 3	4.82	7.03	9.89	13.06	8.70
Mean	3.64	6.51	8.78	10.86	
	SE (m)	CD			
Genotypes	1.46	4.08			
DASI	0.487	1.361			

Table 14: Effect of drought on polyphenol oxidase (activity/min/g) of different black pepper genotypes

Genotype	Polyphenol oxidase (activity/min/g)				
	Control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	5.29	8.88	9.56	10.07	8.45
Aimpiriyan	3.87	5.87	6.66	8.07	6.11
Annavariyan	4.77	6.02	9.27	9.54	7.40
Arikkilazhi	4.87	7.85	8.01	7.93	7.16
Arimulaku	3.77	5.88	6.29	8.10	6.01
Champakkulam local	5.80	7.28	10.10	13.97	9.29
Cholakkodi	4.47	6.58	8.40	9.98	7.36
Karimunda	3.64	6.69	7.59	8.34	6.56
Karuvilanchi	5.10	6.80	7.11	8.60	6.90
Kottinadan	3.19	7.81	8.62	11.16	7.70
Kumbanadan	2.81	4.86	5.31	10.00	5.74
Kuthiravali	4.13	5.74	7.20	9.99	6.76
Malamahar	3.93	6.20	7.01	8.43	6.39
Meenadan	5.27	6.54	7.26	10.26	7.33
Murithothan	4.84	7.46	8.10	11.45	7.96
Narayakodi	2.93	7.64	8.28	9.08	6.98
Nithyakalyani	1.91	5.53	6.26	9.61	5.82
Padappan	4.91	4.79	5.91	7.27	5.72
Palakkodi	4.84	6.73	8.99	9.67	7.56
Pandithitta local	6.65	7.50	6.41	10.73	7.82
Panniyur 1	4.48	7.99	8.36	9.47	7.57
Panniyur 2	4.80	5.92	5.84	8.99	6.39
Panniyur 5	3.32	5.43	6.56	8.29	5.90
Panniyur 6	4.58	6.39	7.16	8.93	6.76
Panniyur 7	4.27	5.86	8.63	10.17	7.23
Panniyur 8	4.26	6.85	7.12	7.27	6.37
Perumkodi	4.76	6.34	6.79	7.95	6.46
Sadanandapuram local	6.08	8.36	8.45	9.88	8.19
Sakthi	4.39	5.52	6.21	7.03	5.79
Subhakara	4.83	8.27	11.30	12.00	9.10
Thekkan	6.08	7.99	7.14	8.31	7.38
Vellamundi	4.41	6.50	0.10	11.89	5.73
Velliyarmunda	4.69	5.03	8.31	7.49	6.38
Wild 1	3.83	6.43	7.82	8.76	6.71
Wild 2	3.87	4.37	4.54	6.61	4.85
Wild 3	2.25	6.44	9.23	10.37	7.07
Mean	4.38	6.56	7.38	9.32	
	SE(m)	CD			
Genotypes	0.787	2.201			
DASI	0.262	0.734			

3.2.7 Glutathione reductase (activity/min/g)

All the genotypes and four stress intensities (control, 5 DASI, 10 DASI and 15 DASI) exhibited significant variation for glutathione reductase content. Highest mean value was recorded by the variety Panniyur 7 (8.10), followed by genotype Pandithitta local (7.86), var. Panniyur 5 (7.49) and Sadanandapuram local (7.36). Lowest mean was recorded by Aimpiriyan (2.17), followed by Wild 3 (2.57) and Wild 1 (2.72). Highest mean concentration was recorded at 15 DASI, followed by 10 DASI and 5 DASI.

Table 15: Effect of drought on glutathione reductase (activity/min/g) of different black pepper genotypes

Genotype	Glutathione reductase (activity/min/g)				
	control	5 DASI	10 DASI	15 DASI	Mean
Adukkanaazhi	3.49	7.01	7.90	9.22	6.90
Aimpiriyan	1.35	2.77	2.28	2.27	2.17
Annavariyan	1.62	6.19	8.02	9.34	6.29
Arikkilazhi	3.57	5.62	4.46	4.61	4.56
Arimulaku	1.70	4.57	4.81	5.47	4.14
Champakkulam local	1.45	2.95	6.61	4.28	3.82
Cholakkodi	3.57	4.02	5.22	6.31	4.78
Karimunda	2.56	4.13	4.24	8.63	4.89
Karuvilanchi	1.18	3.77	4.72	8.59	4.56
Kottinadan	4.03	5.93	6.84	10.24	6.76
Kumbanadan	1.78	2.99	9.61	4.06	4.61
Kuthiravali	2.19	4.91	5.07	8.83	5.25
Malamahar	3.42	6.15	7.64	8.13	6.34
Meenadan	1.40	2.67	3.64	4.80	3.13
Murithothan	4.37	5.68	6.75	12.00	7.20
Narayakodi	4.88	6.91	6.49	7.78	6.51
Nithyakalyani	1.67	4.22	5.85	8.89	5.16
Padappan	4.40	5.44	6.45	6.89	5.80
Palakkodi	2.68	6.99	7.30	9.38	6.58
Pandithitta local	2.49	6.07	11.09	11.80	7.86
Panniyur 1	3.26	4.02	4.99	8.02	5.07
Panniyur 2	4.36	5.15	6.84	9.20	6.39
Panniyur 5	4.74	6.91	8.58	9.75	7.49
Panniyur 6	1.95	2.35	3.18	3.62	2.77
Panniyur 7	2.69	8.23	9.53	11.96	8.10
Panniyur 8	1.95	4.76	7.74	8.58	5.76
Perumkodi	4.91	5.18	6.18	8.71	6.24
Sadanandapuram local	3.47	7.78	8.04	10.18	7.37
Sakthi	3.48	5.94	6.24	7.83	5.87
Subhakara	2.64	2.01	2.63	8.21	3.87
Thekkan	3.75	6.27	7.00	7.82	6.21
Vellamundi	3.39	6.21	6.79	8.57	6.24
Velliyarmunda	2.66	4.68	5.79	8.82	5.49
Wild 1	1.96	2.26	2.72	3.96	2.72
Wild 2	1.33	3.28	5.51	8.24	4.59
Wild 3	1.69	2.09	3.45	3.06	2.57
Mean	2.83	4.89	6.12	7.72	
	SE (m)	CD			
Genotypes	0.89	2.49			
DASI	0.296	0.83			

In general, physiological and biochemical markers for biotic and abiotic stress condition are one and the same. Flooding tolerance is evaluated (quantified) in terms of the growth response, the level of injury sustained and survival in relation to specific flooding characteristics, mainly flooding level (depth) and duration. (Glenz *et al.*, 2006) [8]. Plant responses to submersion vary. They include injury, inhibition of seed germination, changes in vegetative and reproductive growth, changes in plant anatomy, and promotion of early senescence and mortality.

3.3 Selection Index Scores

The selection index technique can theoretically determine the genotypic worth of individuals or families in an objective manner. In general, selection indices provide a useful method for quantifying selection potential as well as providing a good chance for more efficient selection. Selection index was calculated on the basis of all the physiological and biochemical characters viz., relative water content, membrane leakage, proline content, chlorophyll content, peroxidase content, polyphenol oxidase content and glutathione reductase.

Based on these stress responsive characters recorded, selection indices were calculated for all the 36 genotypes for the selection of tolerant ones (Table 16) (Figure 1). Index score was highest for Sadanadapuram local, followed by Pandithitta local, Kottinadan, Adukanazhi, Palakkodi and Murithothan.

Table 16: Selection index scores of 36 genotypes

Individuals	Index scores
Sadanandapuram local	67.9621
Pandithitta local	64.4156
Kottinadan	60.845
Adukkanaazhi	60.2173
Palakkodi	59.5567
Murithothan	59.4912
Nithyakalyani	57.7064
Karuvilanchi	55.9056
Arimulaku	54.3656
Malamahar	53.3944
Subhakara	52.5231
Wild 3	52.5028
Kuthiravali	52.3981
Narayakodi	52.3562
Wild 2	51.8666
Karimunda	51.5358
Velliyarmunda	50.9788
Vellamundi	49.8741
Sakthi	49.7585
Meenadan	48.6586
Annavariyan	47.5188
Wild 1	47.5108
Padappan	47.4538
Champakkulam local	47.1774
Panniyur 1	46.9136
Thekkan	46.709
Panniyur 6	46.5682
Panniyur 8	46.3717
Arikkilazhi	46.0534
Panniyur 2	45.7657
Panniyur 5	44.8757
Perumkodi	43.7283
Panniyur 7	40.5953
Kumbanadan	38.9848
Aimpiriyan	34.8196
Cholakkodi	31.8738



Sadanandapuram local



Pandithitta local

Fig 1: Resistant genotypes identified

4. Conclusion

The evaluation of collected genotypes for drought under artificial conditions was done. Screening for drought tolerance under artificial condition was done in pot culture study in CRD design. Six-month-old rooted cuttings were used for screening and drought was induced by withholding of irrigation for 15 days. Observations on biochemical and physiological parameters including relative water content, membrane leakage, proline content, total chlorophyll, peroxidase, polyphenol oxidase and glutathione reductase activity were recorded at 5 days interval (5, 10 and 15 DAS). A regularly watered control was also maintained. For all the characters significant difference was observed

among the varieties and between different drought intensities i.e., 0, 5, 10 and 15 DAS. Five drought tolerant genotypes were identified from artificial screening viz. Sadanandapuram local, Pandithitta local, Kottinadan, Adukanazhi and Palakkodi.

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