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# Biochemical characterization and genetic variability among thirteen black pepper genotypes

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**There is limited information on the biochemical variability of black pepper genotypes in Ethiopia. Dried fruit samples from 13 introduced black pepper accessions were used for biochemical analysis. All the determinants were done in triplicates. Biochemical data ranged for essential oil between 1.70-3.60%; oleoresin, 8.30-12.15%; ash, 5.72-7.05%; acid insoluble ash, 1.97-4.06%; crude fiber, 13.61-17.96%; moisture, 7.62-10.14%; bulk density, 520.00-738.00 g/L; and piperine, 5.48-5.56%. Accessions differed significantly (P<0.05) in all characters except in oleoresin and piperine. The phenotypic coefficient of variability (PCV) values were relatively greater than genotypic coefficient of variability (GCV), for all traits; however, the GCV values were near to PCV values indicating high contribution of genotypic effect for phenotypic expression of these characters. Heritability in broad sense ($h^2B$) ranged between 66.67-95.24, and genetic advance as percent of the mean (GAM) ranged from 0.79 to 71.84. High heritability coupled with high genetic advance for all characters (except for piperine), reflect the presence of additive gene action for the expression of these characters, and improving of these characters could be done through selection.**

**Key words:** Black pepper, genotypes, biochemical characters, variability, heritability.

## INTRODUCTION

Black pepper (*Piper nigrum* L.) is cultivated for its fruit, which is usually dried and used as a spice and seasoning. The spicy taste is mainly due to the presence of piperine, a pungent alkaloid (Tripathi et al., 1995). It can be used for medicine, human dietaries, preservatives and bio-control agents (Srinivasan, 2007). The essential oil from black pepper possesses antioxidant and antimicrobial activities (Dorman and Deans, 2000).

Knowledge of genetic variability in crop populations is important for selection of superior genotypes. The success of crop improvement program depends on the amount of genetic variation present in a crop and the magnitude of the variation which is heritable from the parent to the progeny (Prasad et al., 1981). Heritability estimate provides information on the extent to which a particular character can be transmitted from the parent to the progeny (Allard, 1991). Genetic advance shows the degree of the gain obtained in a character from one cycle of selection. High genetic advance coupled with high heritability estimates offers the most suitable condition to decide the criteria of selection (Syukur and Rosidah, 2014).

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**Table 1.** Description of thirteen black pepper genotypes.

| S/N | Genotypes | Source country | Year introduced |
|---|---|---|---|
| 1 | Bra.32/79 | Brazilian | 1981/82 |
| 2 | Sri. 3/80 | Srilanka | 1984/85 |
| 3 | Pan. 4/80 | India (kerala) | 1984/85 |
| 4 | Kuch. 5/80 | India (Kuching) | 1984/85 |
| 5 | T4. 17/79 | Costarica | 1981/82 |
| 6 | Pk.-9 | India | 1985/87 |
| 7 | Kw.32 | Srilanka | 1986/87 |
| 8 | DMKW-7 | India | 1986/87 |
| 9 | 15/86 | Srilanka | 1986/87 |
| 10 | Kw.33 | Srilanka | 1986/87 |
| 11 | Kw.11 | Srilanka | 1986/87 |
| 12 | 14/86 | Srilanka | 1986/87 |
| 13 | Gk. 49 | India | 1986/87 |

In Ethiopia, black pepper is part of the daily meal, as a spice and as a source of cash from its sell. It is among spice crops grown for export market. Nevertheless, the supply for both local and export market is limited due to low production and productivity. Knowledge on genetic variability in both morphological and biochemical traits of black pepper genotypes in Ethiopia is limited. Where there is insufficient variation within a crop population of a country or a region, one of the options to increase usable variation is introducing from other regions. The present study was done with the objectives of characterizing biochemically and assessing the magnitude of genetic variability, heritability and genetic advance of thirteen introduced black pepper accessions.

## MATERIALS AND METHODS

### Sample preparation

The biochemical analysis was conducted at Teppi National Spice Research Center (TNSRC) of Ethiopia in July 2015. Samples for the biochemical characterization were taken from 13 accessions of black pepper introduced from different countries (Table 1). The accessions were planted for long term study as the crop is perennial. It was first planted in July 2008. The field design was Randomized Complete Block with three replications. Fruits getting light red were harvested and heaped for about 24 h. For each replication of the treatment 1000 g of fresh berries were collected. The berries were sun dried to 10-12% moisture content on mesh wire bed. The dried samples were prepared for laboratory analysis by grinding as quickly as possible in a grinding mill to pass sieve with 1 mm diameter aperture. The samples were kept in a dry stoppered container until analysis.

### Chemical analysis

The extraction of essential oil from 100 grams of ground sample was done by using hydro-distillation according to AOAC (2000). Oleoresins were determined from thirty grams of ground sample by acetone extract method using Soxhlet apparatus according to AOAC (2000). Ash content was determined from 2g sample by heating the dried end product in furnace at 550$^o$C overnight till the difference between two successive weighing not more than 0.1%, according to AOAC (2000). Acid insoluble ash was determined from the ash using dilute HCl as a solvent according AOAC (2000). Crude fiber was determined from 1.5 g sample using preheated Sulphuric acid ($H_2SO_4$) (1.25% v/v) for acid digestion and natrium hydroxide (NaOH) for alkali digestion according to (AOAC, 2000). Piperine was extracted from 6 g grounded sample using acetone according to AOAC (2000). The concentration was determined by injecting the prepared sample to the High Pressure Liquid Chromatography (HPLC) system. Bulk density and moisture content were determined according to AOAC (2000).

### Data analysis

The analysis of variance (ANOVA) was done using Minitab version 19. Significances were declared at the probability level of 0.05. The variance components: genotypic variance (GV), phenotypic variances (PV) and environmental variance (EV) were determined from mean square values of the ANOVA for each trait according to Prasad et al., (1981). The variance components were used to compute the Genotypic Coefficient of Variability (GCV), Phenotypic Coefficient of Variability (PCV), broad sense heritability ($h^2B$), genetic advance (GA) and genetic advance as percentage of the mean (GAM), according to the methods of Burton (1952), Johnson et al. (1955) and Kumar et al. (1985).

## RESULTS AND DISCUSSION

### Biochemical characterization

The analysis of variance showed that black pepper accessions differed highly significantly ($P<0.001$) in contents of essential oil and bulk density and significantly ($P<0.05$) in contents of acid insoluble ash, crude fiber, ash and moisture content, but not in contents of oleoresin, and piperine (Table 2 and 3). The biochemical data ranged for essential oil between 1.70-3.60%;

**Table 2.** Genotypic and error mean squares from ANOVA for biochemical traits of 13 black pepper accessions.

| S/N | Characters | Genotype mean square (df=12) | Error mean square (df=24) |
|---|---|---|---|
| 1 | Essential oil | 0.87*** | 0.18 |
| 1 | Oleoresin | 3.22 | 1.63 |
| 3 | Ash | 0.77* | 0.11 |
| 4 | Acid insoluble ash | 1.19* | 0.42 |
| 5 | Crude fiber | 7.12* | 3.01 |
| 6 | Moisture | 1.34* | 0.61 |
| 7 | Bulk density | 12582.94*** | 2006.69 |
| 8 | Piperine | 0.001 | 0.001 |

* p<0.05, **p<0.01, ***p<0.001.

**Table 3.** Biochemical composition of 13 black pepper accessions.

| Accession | EO % | Ol% | Ash% | AIA% | CF % | M% | BD g/L | Pip (%) |
|---|---|---|---|---|---|---|---|---|
| 32/79 | 2.15$^{d}$e | 8.97$^{a}$ | 5.93$^{cd}$ef | 3.68$^{ab}$ | 13.77$^{d}$ | 10.14$^{a}$ | 728.67$^{a}$ | 5.51$^{a}$ |
| 17/79 | 2.95$^{abc}$ | 9.22$^{a}$ | 5.92$^{d}$ef | 1.97$^{d}$ | 14.77$^{bcd}$ | 9.17$^{abc}$ | 732.33$^{a}$ | 5.51$^{a}$ |
| 3/80 | 2.40$^{bcd}$e | 8.87$^{a}$ | 6.30$^{cd}$e | 3.75$^{ab}$ | 17.28$^{ab}$ | 8.01$^{cd}$ | 727.33$^{a}$ | 5.53$^{a}$ |
| 4/80 | 2.20$^{d}$e | 9.65$^{a}$ | 6.32$^{cd}$ | 3.35$^{abc}$ | 16.06$^{abcd}$ | 8.92$^{abcd}$ | 729.67$^{a}$ | 5.53$^{a}$ |
| 5/80 | 2.35$^{cd}$e | 9.70$^{a}$ | 5.74ef | 3.18$^{abc}$ | 14.10$^{cd}$ | 7.62$^{d}$ | 663.33$^{abc}$ | 5.52$^{a}$ |
| PK/9 | 3.10$^{ab}$ | 8.30$^{a}$ | 6.48$^{bc}$ | 3.81$^{ab}$ | 13.61$^{d}$ | 9.63$^{ab}$ | 713.33$^{ab}$ | 5.48$^{a}$ |
| 14/86 | 2.05e$^{d}$ | 10.55$^{a}$ | 5.86$^{d}$ef | 3.14$^{abc}$ | 17.96$^{a}$ | 8.47$^{bcd}$ | 650.67$^{bc}$ | 5.52$^{a}$ |
| 15/86 | 2.70$^{bcd}$ | 11.10$^{a}$ | 7.02$^{ab}$ | 3.00$^{abcd}$ | 14.36$^{bcd}$ | 8.85$^{abcd}$ | 722.00$^{ab}$ | 5.50$^{a}$ |
| KW/11 | 1.70e | 10.37$^{a}$ | 5.72f | 2.72$^{bcd}$ | 14.20$^{cd}$ | 9.05$^{abc}$ | 738.00$^{a}$ | 5.50$^{a}$ |
| KW/32 | 3.60$^{a}$ | 10.22$^{a}$ | 6.89$^{ab}$ | 4.04$^{a}$ | 16.98$^{abc}$ | 9.29$^{abc}$ | 616.67$^{c}$ | 5.56$^{a}$ |
| KW/33 | 2.05e$^{d}$ | 10.00$^{a}$ | 5.73f | 2.76$^{bcd}$ | 16.37$^{abcd}$ | 8.34$^{bcd}$ | 734.33$^{a}$ | 5.56$^{a}$ |
| DMKW/7 | 2.15e$^{d}$ | 10.75$^{a}$ | 7.05$^{a}$ | 4.06$^{a}$ | 13.66$^{d}$ | 8.44$^{bcd}$ | 520.00$^{d}$ | 5.51$^{a}$ |
| GK/49 | 2.99$^{abc}$ | 12.15$^{a}$ | 5.77$^{d}$ef | 2.52$^{cd}$ | 14.08$^{cd}$ | 8.80$^{bcd}$ | 732.67$^{a}$ | 5.51$^{a}$ |

EO %= Essential oil %, Ol%=Oleoresin%, AIA% = Acid insoluble ash, CF %=Crude fiber %, M%=Moisture%, BD g/L =Bulk density g/L, Pip(%)= Piperine (%); Means in a column that do not share a letter are significantly different.

oleoresin, 8.30-12.15%; ash, 5.72-7.05%; acid insoluble ash, 1.97-4.06%; crude fiber, 13.61-17.96%; moisture, 7.62-10.14%; bulk density, 520.00-738.00 g/L; and piperine, 5.48-5.56% (Table 3). The result shows presence of a considerable variation in biochemical characters among the accessions.

## Analysis of variance components

Estimates of the variance components: phenotypic variances (PV), genotypic variances (GV) and environmental variance (EV) are shown in Table 4. The result showed that the phenotypic variances are higher than the genotypic variances. Nevertheless, the PV and GV values are close to each other. The genotypic variances were greater than the error variances for all traits (Table 4). These high genotypic variances indicate that the genotypic component was the major contributor to the total variance for these biochemical traits. Similar results have been reported by other workers (Baye, 2002; Nwofia and Chilekwe, 2015).

Similarly, the PCV values were relatively greater than GCV for all traits; however, the GCV values were near to PCV values (Table 4). The closeness of the values indicates that there was little influence of the environment in the expression of these traits, suggesting the presence of high genotypic contribution for phenotypic expression of these characters (Nwofia and Chilekwe, 2015). It can be concluded that most of the variability observed in the biochemical traits of black pepper are more of a genetic than environmental basis. This indicates that there is substantial opportunity for selection among the accessions for the biochemical traits.

The success of crop improvement program depends on the amount of heritable genetic variation present in a crop (Prasad et al., 1981). Hence heritability is used by breeders in exercising the reliability of phenotypic values. The genetic advance indicates the progress that can be anticipated as a result of exercising selection on the

**Table 4.** Estimation of components of variance, coefficients of variation, heritability and genetic advance for the biochemical traits of 13 black pepper accessions.

| S/N | Character | Mean | EV | GV | PV | GCV | PCV | $h^2B$ | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Essential oil | 2.49 | 0.18 | 0.81 | 0.87 | 36.14 | 37.46 | 93.10 | 1.79 | 71.84 |
| 2 | Oleoresin | 9.98 | 1.63 | 2.68 | 3.22 | 16.39 | 17.98 | 83.13 | 3.07 | 30.79 |
| 3 | Ash | 6.21 | 0.11 | 0.73 | 0.77 | 13.79 | 14.13 | 95.24 | 1.72 | 27.72 |
| 4 | Acid Insoluble Ash | 3.23 | 0.42 | 1.05 | 1.19 | 31.72 | 33.77 | 88.24 | 1.98 | 61.39 |
| 5 | Crude Fiber | 15.17 | 3.01 | 6.12 | 7.12 | 16.30 | 17.59 | 85.91 | 4.72 | 31.13 |
| 6 | Moisture | 8.82 | 0.61 | 1.14 | 1.34 | 12.09 | 13.12 | 84.83 | 2.02 | 22.93 |
| 7 | Bulk Density | 693 | 2006.69 | 11914.04 | 12582.94 | 15.75 | 16.19 | 94.68 | 218.79 | 31.57 |
| 8 | Piperine | 5.52 | 0.00 | 0.00 | 0.00 | 0.47 | 0.57 | 66.67 | 0.04 | 0.79 |

Mean = grand mean for the character, EV= environmental variance, GV = genotypic variance, PV = phenotypic variance, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, $h^2B$ = broad sense heritability, GA = genetic advance, GAM = genetic advance as percentage of the mean.

population (Sood et al., 2011). Heritability was high for all biochemical traits (Table 4), indicating low influence of the environment on the expression of these traits. Heritability along with genetic advance is more useful than heritability alone in predicting the subsequent effect of selecting better performing individual genotypes as it suggests the presence of additive gene effects (Syukur and Rosidah, 2014).

Heritability in broad sense ($h^2B$) ranged between 66.67-95.24, and genetic advance as percentage of the mean (GAM) ranged from 0.79-71.84 (Table 4). High heritability coupled with high genetic advance as percentage of the mean for all characters except for piperine, reflecting the presence of additive gene action for the expression of these characters, and improving of these characters could be done through selection.

## Conclusion

The results from the analysis of variance showed significant variability between accessions of black pepper for essential oil, ash, acid insoluble ash, crude fiber, moisture, and bulk density. The presence of the variability was further evidenced by the estimates of variance components. The obtained variability was more of genetic than environmental. High heritability and genetic advance observed for all characters indicate that these biochemical characters are controlled by additive genes and selection can be effective for their improvement.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.


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