



Impact of Shade and Light Conditions on Growth, Yield and Chemical Contents in *Curcuma caesia* Roxb

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ABSTRACT

Background: The growth, yield and chemical contents of the plants are affected by biotic and abiotic stresses. Plant growth, yield and chemistry were always strongly affected by lighting conditions. Different genotypes tend to have distinct genetic makeup resulting in varying growth characters.

Methods: The main objective of the study was to evaluate the difference in growth, yield and chemical contents in *Curcuma caesia* under open and shade conditions. Four *C. caesia* genotypes were collected from different parts of North East India and grown in open and 50% shade environments in the farm of College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh. Observations were recorded for various morphological and yield characters. The phytochemical content of the rhizomes grown in both open and shaded environments were analyzed using GCMS.

Result: It was found that the four genotypes have significant differences in the morphological and yield characters. Significant interaction was found between the genotypes and shade conditions for leaf area and rhizome yield. The genotype G4 performed best in terms of yield of rhizomes and the condition S1 is found to be the best. G4S1 outperformed the others in terms of yield when considering the interaction effect between genotype and condition.

Key words: Black haldi, Environmental factor, GCMS, Rhizome.

INTRODUCTION

Curcuma caesia Roxb. is popularly known as 'Black Turmeric' is a perennial rhizomatous herb having high medicinal values. Taxonomically, it is placed within the order *Zingiberales*, the family *Zingiberaceae*, the subfamily *Zingiberoideae*, the tribe *Hedychieae* and the genus *Curcuma*, with the specific species identified as *Curcuma caesia* Roxb. Schoch *et al.*, 2020; Pandey *et al.*, 2022). The plant is a native to East, central and North Eastern part of India. This endangered herb is also found in other Southeast Asian countries and China (Ibrahim *et al.*, 2023). The leaf vein is blackish in colour and the inner part of the matured rhizome is bluish in colour. The plant can be cultivated round the year but it goes into hibernation and leaves turned yellowish brown and fell off during the winter season. It has been reported to have extensive medicinal values and have much use in traditional healing practices. It has traditional uses, such as for treating leucoderma, asthma, tumors and bruises and has versatile medicinal properties (Mahato and Sharma, 2018). It is reported to have anticancer activity (De Cicco *et al.*, 2018), reduce endothelial dysfunction (Yamagata *et al.*, 2020), hyperglycemia (Hashim *et al.*, 2018), anti-inflammatory, antibacterial and antioxidant (Amalraj *et al.*, 2017). Kanglom *et al.*, (2023) reported that this plant has antioxidants and antimicrobial activities. Kumar *et al.*, (2022) reported that rhizome extracts inhibit the growth of Gram positive bacteria like, *Bacillus cereus*, *Bacillus subtilis* and *Streptococcus agalactiae*. The study conducted by Das *et al.* (2012) demonstrated that the ethanol extract of *C. caesia* rhizome reduce gastric acid, free acidity while

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simultaneously enhancing mucus production. Additionally, people use the plant as a supplement in the form of a tonic or directly consume the rhizomes to cure different diseases such as asthma, cough, cold, bronchitis, lung issues, diarrhoea and dysentery. Moreover, these rhizomes are also used to treat internal wounds and are believed to aid blood clotting post surgery (Paudel *et al.*, 2024). It is also reported that women on post childbirth consume them to heal surgical wounds (Venugopal *et al.*, 2017). Borah *et al.*, (2020) reported the plant having many properties like antitumor, antioxidant, antimicrobial, thrombolytic, antiasthmatic, wound healing, treating fever, dysentery, stomachache, hemorrhoids, epilepsy, menstrual disorder, cancer, antihelminthic, leprosy, aphrodisiac, asthma, piles,

leukoderma, hemorrhoids, inflammation and bronchitis. However, despite its local acclaim, the medicinal potential of *C. caesia* remains unexplored scientifically, leaving numerous promising therapeutic benefits untapped and unverified (Shahinozzaman *et al.*, 2013).

Major components in essential oil of *C. caesia* are found to be linalool followed by ocimene, 1- ar-curcumene, zingiberol, 1, 8-cineole and borneol; Eucalyptol (28.55%), camphor (21.73%) and epicurzerenone (19.62%) identified as major components in the rhizome essential oil of *C. caesia* (Lal *et al.*, 2022). The volatile oil is claimed to comprise 30 components with camphor, ar-turmerone, (Z)- β -ocimene, ar-curcumin, 1, 8-cineole, β -element, borneol, bornyl acetate and γ -curcumene as the major constituents (Chaturvedi *et al.*, 2019). The three primary curcuminoids namely demethoxycurcumin, curcumin and bisdemethoxycurcumin are found to be present in this plant (Amalraj *et al.*, 2017). The GC-MS analysis showed that the major components present in the *C. caesia* were α -Santalol (46.90%), Retinal (10.72%), Megastigma-3,7(E),9-triene, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl(4.38%), 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- (4.26%) tricyclo [8.6.0.0(2, 9)] hexadeca-3, 15-diene, trans-2, 9-anti-9, 10- trans-1, 10(3.26%) and various other compounds were identified as low level (Muthukumaran *et al.*, 2017). Borah *et al.*, (2020) also reported the major compounds in *C. caesia* as Androsta-1,4-dien-3-one,17(acetyloxy)-, (17.beta.)-Santanol acetate (16.11%), Eucalyptol (12.98%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) (8.96%), methyl 7,12-octadecadienoate (6.75%) and (+)-2-Bornanone (6.60%). Atom *et al.*, (2023) found that all the trace elements namely Na, K, Mg, Fe, Ca, Co, V, Cr, Ni, As, Mn, Cu and Pb found in the rhizomes of *Curcuma caesia* were within the permissible limits of FAO and WHO and thus rhizomes can be considered as potential source for nutritional as well as herbal formulations.

Generally, plant growth and development are influenced by genetic and environmental factors. While the genetic factor usually determines certain physical features of a plant, the expression of genes is greatly influenced by environment (Brown *et al.*, 2014). *C. caesia* is a partial shade loving plant but can also be grown in open conditions. Bioproduction and accumulation of secondary metabolites in medicinal plants are generally affected by environmental factors, such as temperature, water, light and soil properties (Verma and Shukla, 2015; Li *et al.*, 2020). Due to variations in light conditions, different regions have distinct secondary metabolite compositions and contents. (Huang and Liu, 2015). Comparative analysis on the plant growth, yield and chemical contents under open and shade conditions will be very useful in developing its proper cultivation practices and higher rhizome yield through conventional and non conventional methods. Half strength of MS basal medium containing BAP (1.0 mg/l) and 9% sucrose was found to be optimum for induction of large sized microrhizome within 45 days of incubation under 16 hrs of photoperiod (Sarma *et al.*, 2021). Systematic cultivation and crop improvement work on this species is at a very budding stage though the demand of this species is gradually increasing for its high medicinal values. Survey, collection and documentation of different genotypes are urgently needed to identify the genotypes with high rhizome yield and high bioactive content. Considering the above points, four genotypes which were collected from various parts of North East India were grown under open and 50% shade conditions to evaluate the growth, yield and chemical contents.

MATERIALS AND METHODS

The present investigation was carried out at College of Horticulture and Forestry (CHF), Central Agricultural University (CAU), Pasighat situated at the foothills of Arunachal Pradesh, India (N 28°3'42.8364", E 95°19' 33.3696") with an elevation of 150 meters above MSL (Fig 1).

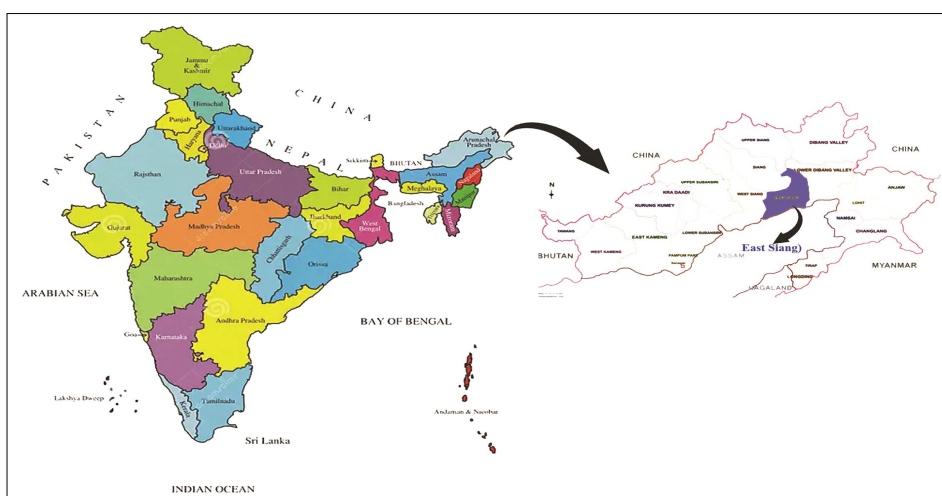


Fig 1: Location of the study, East Siang district, Arunachal Pradesh.

Four genotypes of *C. caesia* were collected from various parts of NE India (Fig 2). The collections were maintained in the research field at CHF, CAU, Pasighat, Arunachal Pradesh. The Indigenous Collection (IC) numbers were acquired from NBPGR, New Delhi. The four genotypes (G1-IC-0644025, G2 (IC-0644026, G3 (IC-0644027 and G4-IC-0644028) were grown under 50% shade (S1) and open conditions (S2). Biometrical observations were recorded at maturity and the chemical constituents of the rhizomes for each genotype grown in shade and open the chemical analysis was done using Gas Chromatography Mass Spectrometry (GCMS). The crop was planted in the field in the month of March-April, 2022 and was ready to harvest by January, 2023. the crop is generally harvested after 2 years as it gives the maximum yield and good quality rhizomes. Various data on morphological parameters like plant height (cm), leaf breadth (cm), leaf length (cm), leaf area (cm²), petiole length (cm) and rhizome yield (kg) of the plants were recorded for 2 years. Five plants per treatment for each genotype from each replication were specified for recording the parameters.

GCMS analysis of the rhizomes of the genotypes was carried out as per Srikanth *et al.* (2022) with some modifications. The peaks acquired by GC-MS were identified by comparing their mass spectra with NIST library mass spectra's using Agilent mass hunter workstation qualitative analysis software, version 10.0 (Srikanth *et al.*, 2022).

Statistical analysis of data was done using the R Studio software ver.4.2.2. Variations arises among the treatments were calculated by Least Significant Difference (LSD) values at 5% probability. Principal coordinate Analysis

(PCA) was also carried out for the chemical constituents and the genotypes using the PAST 3 software.

RESULTS AND DISCUSSION

Morphological characters

Significant differences were observed for growth and yield characters in four genotypes in shade and open conditions.

Effect of Genotypes

The effect of genotypes was significant for leaf area, leaf length, plant height, petiole length and rhizome yield (RY). Plant height was maximum in G3 with 105.67 cm which was on par with G4 (99.00 cm) and lowest in G1 (89.33 cm). Highest leaf length was recorded in G3 (52.00cm) which was on par with G2 and G4 (49.83 cm and 45.17 cm respectively). Lowest leaf length was recorded in G1 (40.75 cm). G4 showed the highest leaf area with 438.00 cm² which was on par with G3 (377.67 cm²) and lowest in G2 (279.83 cm²). G3 recorded the highest petiole length (27.50 cm) and the lowest in G4 (19.17 cm). G4 with 9.90 kg recorded the highest in RY and the lowest in G1 (3.49 kg) (Table 1).

Effect of shade

The effect on shade showed significant values for plant height, leaf breadth, leaf length, leaf area, petiole length and yield of rhizomes. All the characters under study performed best in shade (S1) in contrast with open (S2) condition. S1 with 122.83 cm emerged highest in the plant height in contrast with S2 (70.58 cm); Leaf breadth was 11.71 cm in S1 while it is 11.00 cm in S2; Leaf length was highest in S1 (60.13 cm) in contrast with S2 (35.80 cm); Leaf area was the highest in S1 (473.25 cm²) in contrast



Fig 2: The 4 genotypes of *C. caesia* under study.

with S2 (240.67 cm²); Petiole length recorded highest in S1 (26.42 cm) while it was 19.42 cm in S2 condition and RY was highest in S1 (8.79 Kg) in contrast with 2.79 Kg in Open condition (Table 1).

Interaction effect of genotypes and shade

The interaction effect of genotype and shade condition showed significant result in leaf area and rhizome yield. Highest leaf area was recorded in G4S1 (634.33 cm²) and the lowest in G1S2 (221.33 cm²). G4S1 (16.27 kg) recorded the highest rhizome yield and the lowest in G1S2 (1.90 kg) (Table 1).

Srikrishnah *et al.* (2012) also recorded significant higher leaf area, biomass and yield in shades of 50% and 70% in contrast with the 0% shade levels in 3 varieties of *Dracaena sanderiana* L. The different varieties of ginger performed better in terms of morphological and yield characters and rhizome yield under shade conditions in contrast with the open conditions (Babu *et al.*, 2019). The increase in fresh weight of rhizome might be attributed to higher photosynthetic activity under favourable soil

temperature, low light intensity and higher relative humidity under shade net condition. The increased in plant height, leaf sizes, leaf area per plant might eventually affect the rhizomes characters which resulted in a higher yield of rhizome in shade condition in contrast with plants grown in open conditions. Generally, the growth response of plants in shade might also be strictly depended on genotype and thus is species-specific (Fini *et al.*, 2010). *M. melissophyllum*, which a typical shade loving plant when grown in direct sunlight produced more number of shoots than those grown in shade. The shoots were shorter and the leaf area was smaller than those plants grown in shade condition (Sandhu *et al.*, 2020). When a plant is subjected to acclimatize to inhospitable conditions, the structure and diameters of the leaves may change (Anderson *et al.*, 1991; Cai *et al.*, 2007; Van Hees *et al.*, 2003). Typically, when grown in deep shade, the leaf area expands to enhance light absorption, while its thickness and mass per unit decrease (Hirano *et al.*, 2019). Budiastuti *et al.* (2022) also reported highest number of leaves, fresh plant weight and total biomass

Table 1: Morphological characters and yield of 4 genotypes grown under shade and open conditions.

Genotypes	Plant height (cm)	Leaf breadth (cm)	Leaf length (cm)	Leaf area (cm ²)	Petiole length (cm)	Rhizome yield (Kg)/Kg of planting material used
G1	89.33	10.70	40.75	332.33	22.83	3.49
G2	92.83	11.75	49.83	279.83	22.17	5.06
G3	105.67	11.17	52.00	377.67	27.50	4.72
G4	99.00	11.83	45.17	438.00	19.17	9.90
SEm±	2.67		2.57	26.47	1.20	0.04
SEd±	3.77		3.64	37.43	1.70	0.06
CV(%)	6.75		13.43	18.16	12.86	1.73
LSD _G	8.09	NS	7.81	80.28	3.65	0.12
Shade condition						
S1	122.83	11.71	60.13	473.25	26.42	8.79
S2	70.58	11.00	33.75	240.67	19.42	2.79
SEm±	1.87	0.23	1.82	18.71	0.85	0.03
SEd±	2.67	0.32	2.57	26.47	1.20	0.04
CV(%)	6.75	6.88	13.43	18.16	12.86	1.73
LSD _S	5.72	0.68	5.52	56.77	3.65	0.09
GxS						
G1S1	118.00	11.40	58.83	443.33	26.33	5.07
G2S1	116.33	11.83	58.33	314.67	25.67	6.92
G3S1	135.33	11.33	63.67	500.67	31.00	6.90
G4S1	121.67	12.67	59.67	634.33	22.67	16.27
G1S2	60.67	10.00	22.67	221.33	19.33	1.90
G2S2	69.33	11.67	41.33	245.00	18.67	3.20
G3S2	76.00	11.00	40.33	254.67	24.00	2.53
G4S2	76.33	11.00	30.67	241.67	15.67	3.53
SEm±				37.43		0.06
SEd±				52.94		0.08
CV(%)				18.16		1.73
LSD _{GxS}	NS	NS	NS	113.53	NS	0.17

in *Indigofera tinctoria* L. were observed under the shading treatment applied during the early growth stage (up to one month after planting) at 50% light intensity. Maximum indican production, reaching 843.33 ppm, was recorded under mid growth shading (up to two months after planting) at 10% light intensity. Thus the result in our experiment indicated the effect of genotype and shade conditions on the growth and yield of the plants.

Chemical constituents

GCMS analysis was done for the samples collected from the genotypes grown under shade and open detected. Results indicated a quite difference in the chemical content among the genotypes and between the same genotype grown under shade and open conditions. The expression of compounds in shade and open conditions vary in numbers and types and also amongst the genotypes the expressions of compounds were different. Those compounds which were expressed commonly in shade and open conditions were compared. In G1, a total number of 52 commonly expressed compounds were analysed and their peaks were compared (Fig 3). In G2, a total number of 34 commonly expressed compounds were analysed and their peaks were compared (Fig 4). In G3, a total number of 38 commonly expressed compounds were analysed and their peak heights were compared (Fig 5). In G4, a total number of 32 commonly expressed compounds were analysed and their peaks were compared (Fig 6).

A total of 18 compounds were identified which were expressed commonly in all the genotypes grown under shade and dry conditions. The commonly expressed compounds were Heptadecane, Lyxose, O,O,O,O-TMS MEOX1, Piperidine hydrochloride, Ribitol TMS, Myo-Inositol, 6TMS derivative, Iron, tricarbonyl [N-(phenyl-2-pyridinylmethylene)benzenamine-N,N']-, Epicurzerenone, Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-Hexanol-4-D2, Trehalose TMS, Phosphonic acid, dioctadecyl ester, Palmitic Acid, TMS derivative, Stearic acid, TMS derivative, 3,4-Pentadien-2-d-1-ol, Octyl (t-Butyl) Carbonate, trans-2-methyl-4-n-pentylthiane, S,S-dioxide, 2,3-Dimethylthiirane 1,1-dioxide, .beta.-Vetivenene. The various peak comparisons of these compounds are given in (Fig 7).

The bioproduction of terpenoids in plants is closely linked to light conditions and both ultraviolet (UV) and regular light serve as significant triggers for terpenoid synthesis (Zhang and Bjorn, 2009; Kawoosa *et al.*, 2010; Xie *et al.*, 2021). Among various light forms, UV-B is found to enhance terpenoid accumulation in many plant species (Takshak and Agrawal, 2019). Phenolic accumulation in plants is influenced by several factors, with genotype, ontogeny and environmental conditions related to biotic and abiotic factors being the most critical (Verma and Shukla, 2015). It has been demonstrated that shading reduces the concentration of phenolics and alters their composition in the berry skins of *Vitis vinifera* cv. Cabernet

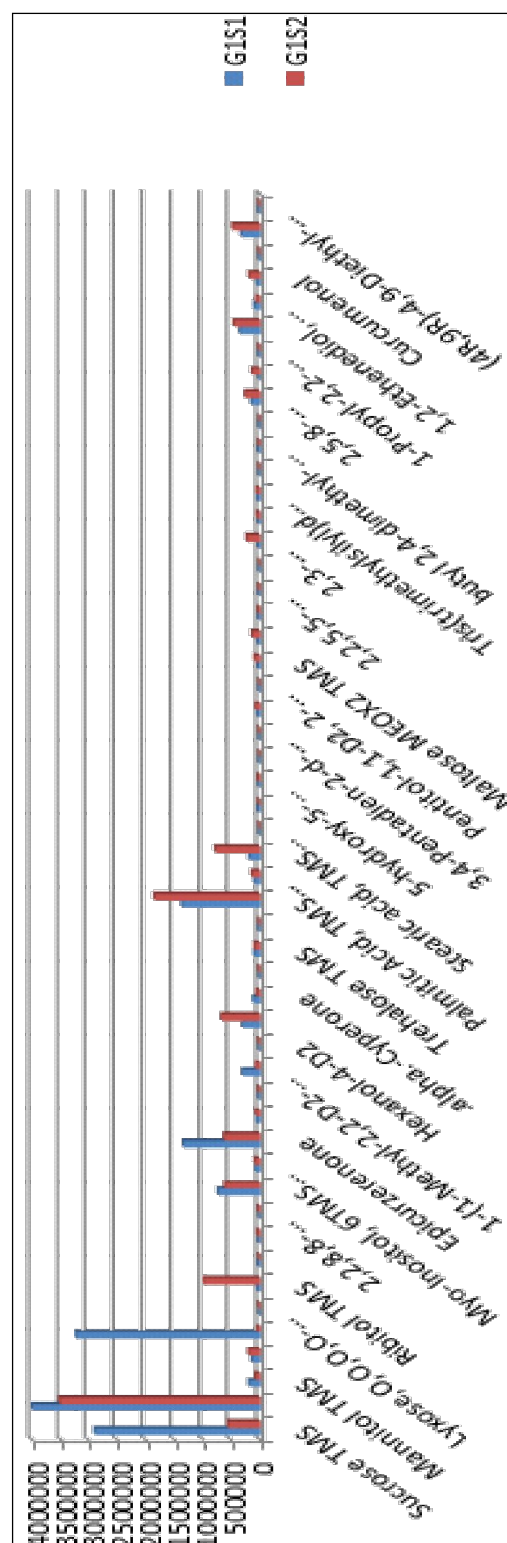


Fig 3: Graph showing the expression of different chemicals in G1 grown under shade and open conditions.

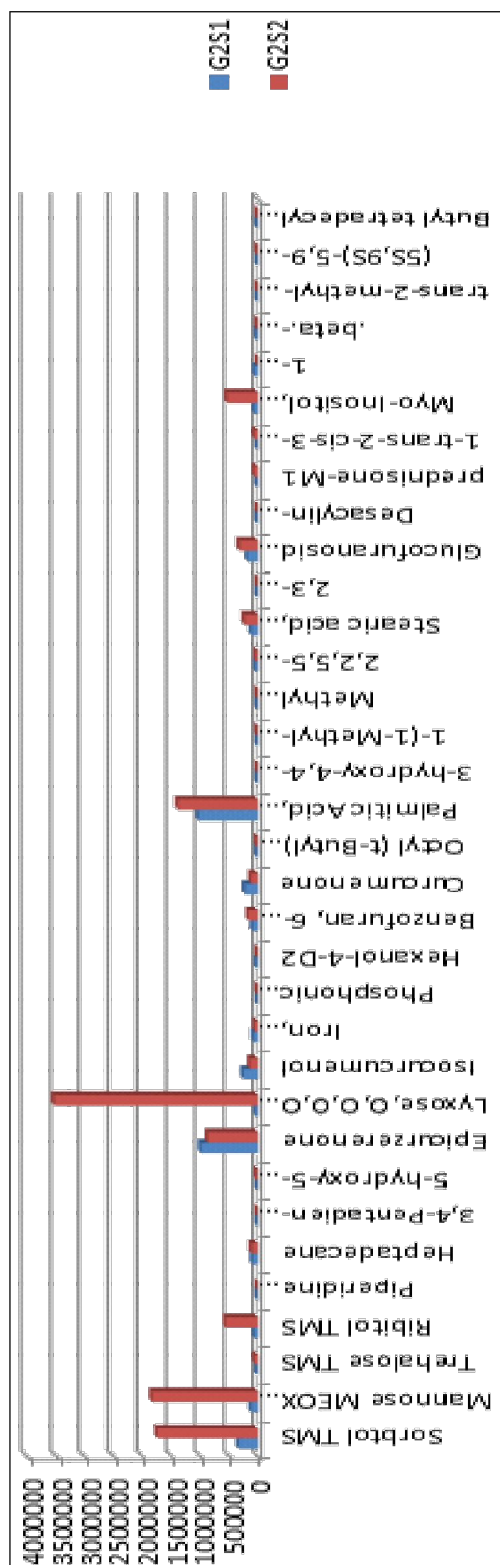


Fig 4: Graph showing the expression of different chemicals in G2 grown under shade and open conditions.

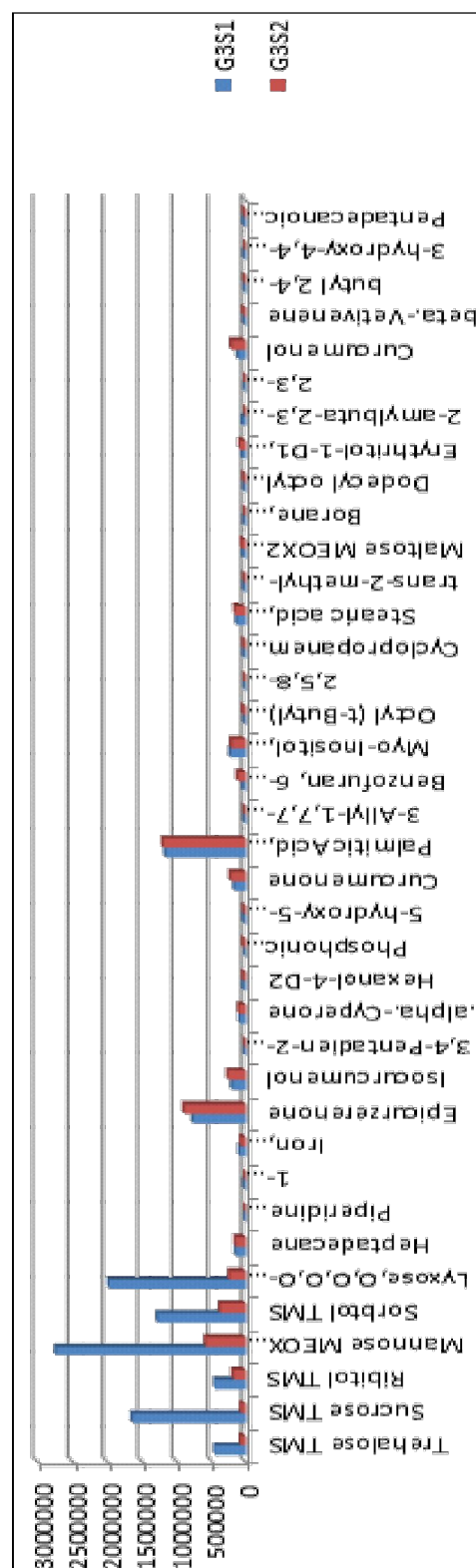


Fig 5: Graph showing the expression of different chemicals in G3 grown under shade and open condition.

Sauvignon (Koyama *et al.*, 2012). Therefore, light levels may be an effective tool for modifying the chemical profile in plants. In many species, higher doses of photosynthetically active radiation (PAR), within the 400-700 nm range, have been shown to stimulate the synthesis of flavonoids and hydroxycinnamic acids. This effect has been particularly well-documented in shade-loving plants such as bilberry, *Mikania laevigata* Sch. Bip. ex Baker and *Mikania glomerata* Spreng. Additionally, it has been reported that the coumarin content in the leaves of *M. laevigata* increases with the level of shade (Bertolucci *et al.*, 2013). A similar trend was observed in *Hierochloa australis* (Schrud.) Roem. et Schult. (Bączek *et al.*, 2019). Both species are typical undergrowth plants, found in semi-shaded environments where the coumarin accumulation varies under different light conditions.

Principal component analysis (PCA)

Principal component analysis was carried out separately for the four genotypes (G1, G2, G3 and G4) grown under shade and open conditions.

The experimental dataset consists of 2 treatment combinations for 52 respective variables (Compounds expressed under GCMS) for G1 genotypes in the analysis. Analysis was conducted with all 52 variables together and the loadings plot and the component matrix were used to interpret the dataset. The loading plot shows the correlation with principal component and the original variables. Principal components i and ii account for 86.83% and 13.16 %, respectively, of the total variation in the dataset, so the two-dimensional loading plot of the data set given in the figure is an excellent approximation to the original scatter-plot in two-dimensional space. Among all the loadings, Mannose MEOX TMS, Lyxose, O,O,O,O-TMS MEOX1 and Sucrose TMS has contributed majorly under PC¹ while Mannose MEOX TMS, Palmitic Acid, TMS derivative and Ribitol TMS has contributed majorly under PC² (Fig 8a).

For the genotype G2, the experimental dataset consists of 2 treatment combinations for 34 respective variables (Compounds expressed under GCMS) in the analysis. All the 34 variables together and the loadings plot and the component matrix were used to interpret the dataset. The loading plot shows the correlation with the principal components and the original variables. Principal components I and II account for 91.89% and 8.11 % of the total variation in the dataset, so the two-dimensional loading plot of the data set given in the figure is an excellent approximation to the original scatter plot in two-dimensional space. Among all the loadings, Palmitic Acid, TMS derivative, Epicurzerenone and Sorbtol TMS has contributed majorly under PC¹ while Lyxose, O,O,O,O-TMS MEOX1, Mannose MEOX TMS, Sorbtol TMS has contributed majorly under PC² (Fig 8b).

The experimental dataset consists of 2 treatment combinations for 38 respective variables (Compounds expressed under GCMS) for G3 genotypes in the analysis. All the 38 variables together and the loadings plot and the

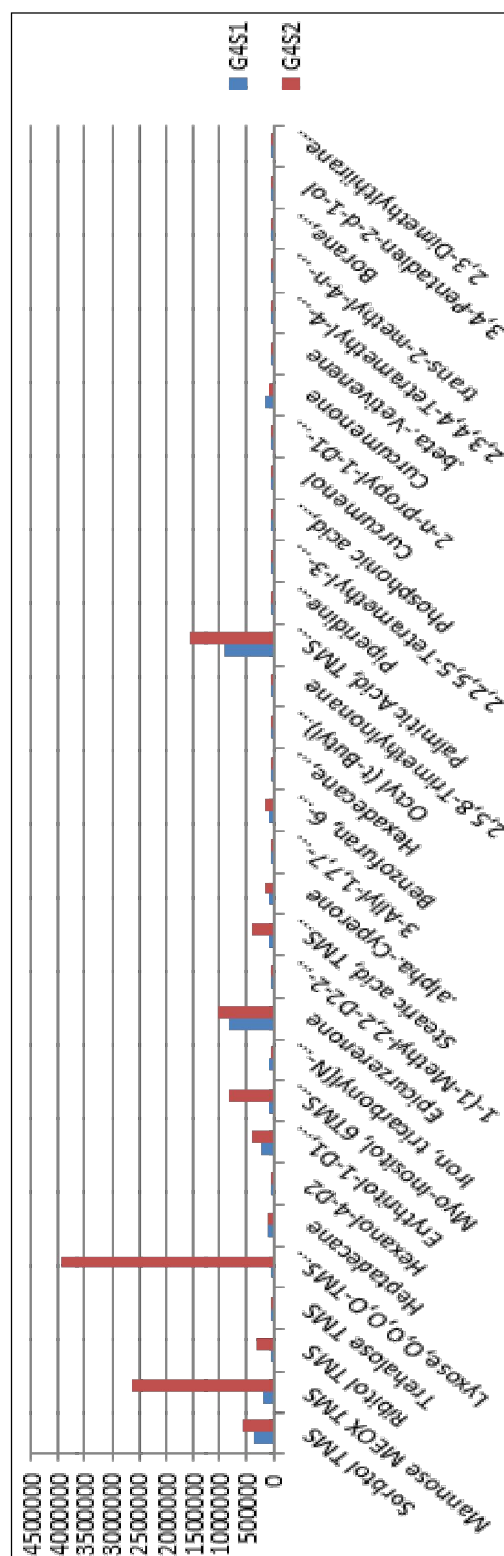


Fig 6: Graph showing the expression of different chemicals in G4 grown under shade and open conditions.

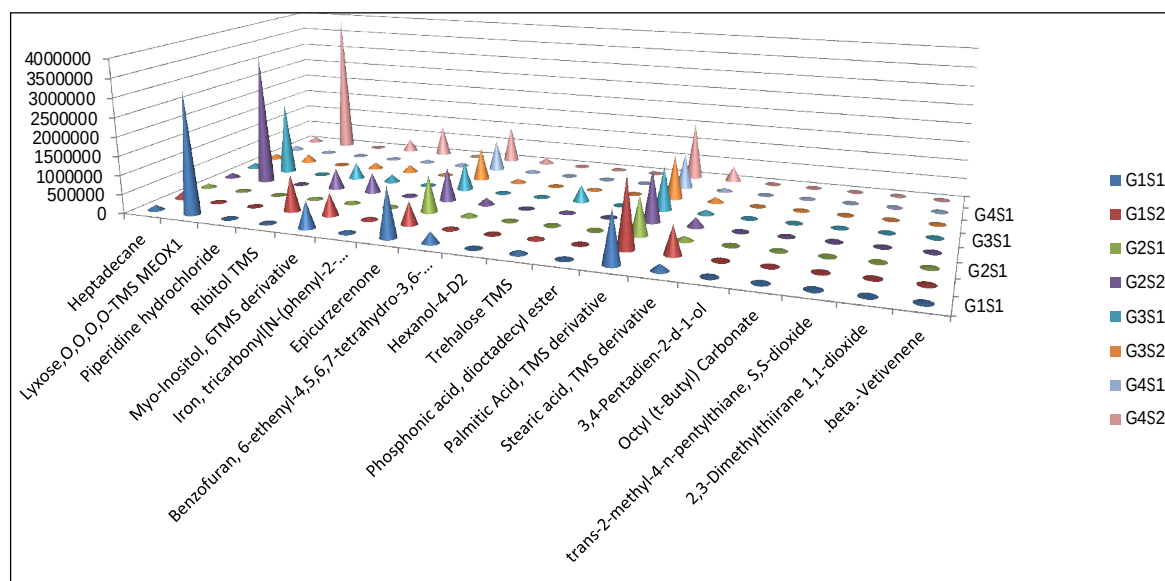


Fig 7: Graph showing the peaks of different commonly expressed chemicals of the four genotypes grown under shade and open conditions.

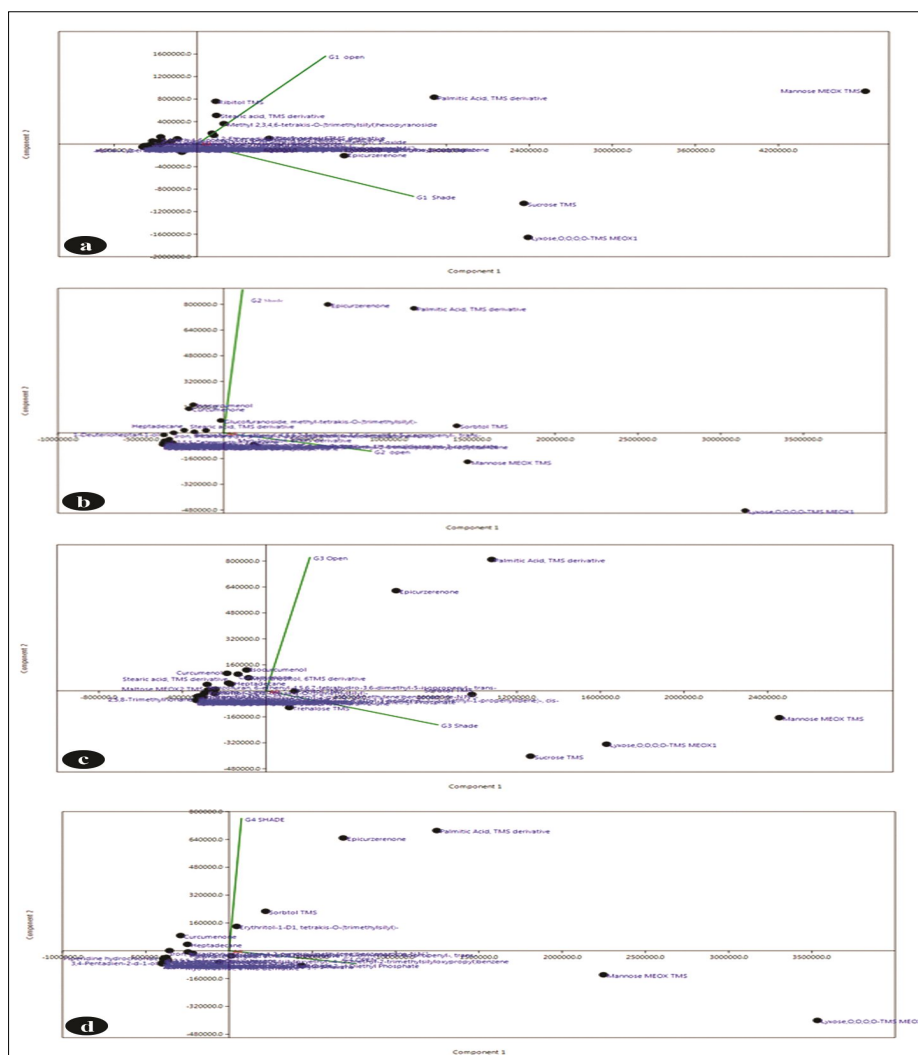


Fig 8: Principal component analysis (PCA) of the 4 genotypes and the chemicals expressed grown in shade and open conditions.

component matrix were used to interpret the dataset. The loading plot shows the correlation with principal components and the original variables. Principal components I and II account for 91.36% and 8.63 %, respectively, of the total variation in the dataset, so the two-dimensional loading plot of the data set given in the figure is an excellent approximation to the original scatter-plot in two-dimensional space. Among all the loadings, Mannose MEOX TMS, Lyxose, O, O, O, O-TMS MEOX1 and Sucrose TMS has contributed majorly under PC1 while Palmitic Acid, TMS derivative, Epicurzerenone, Mannose MEOX TMS has contributed majorly under PC2 (Fig 8c).

The dataset of G4 consists of 2 treatment combinations for 32 respective variables (Compounds expressed under GCMS) in the analysis. All the 32 variables together and the loadings plot and the component matrix were used to interpret the dataset. The loading plot shows the correlation with principal components and the original variables. Principal components I and II account for 94.86 % and 5.13 %, respectively, of the total variation in the dataset, so the two-dimensional loading plot of the data set in the figure is a remarkable approximation to the original scatter-plot in two-dimensional space. Among all the loadings, Palmitic Acid, TMS derivative, Epicurzerenone and Sorbitol TMS has contributed majorly under PC1 while Lyxose, O, O, O, O-TMS MEOX1, Mannose MEOX TMS, Palmitic Acid, TMS derivative has contributed majorly under PC2 (Fig 8d).

CONCLUSION

The findings of our experiment indicated the variant response of different genotypes when grown under shade and open conditions in terms of morphological growth, rhizome yield and chemical contents. The study revealed that the genotype G4 performed well in terms of rhizome yield and the plants performed better in shade condition as compared to open condition. G4S1 had the highest rhizome yield in the genotype-shade interaction. The morphological characters and rhizome yield of the four genotypes were influenced when grown under shade and open conditions. The morphological parameters and rhizome yield was found to be more in plants grown under shade conditions in contrast with the plants grown in open conditions. This must be with habitat of shade-loving plants under study. GCMS analysis revealed that there is variation in the chemical content of the different genotypes and the expression of chemicals of the genotypes varies when grown under shade and open conditions. The expression of important chemicals under different shades can be studied further for the cultivation of the crop under the optimal shade conditions for better rhizome and chemical yield. Study on more numbers of genotypes collected from different climatic conditions will be an important aspect in this field.

Conflict of Interest

The authors declare that there is no conflict of interest.

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