Differential response of *Zingiber officinale Rosc.* Cv. varada to solitary and combined stress treatments with respect to yield, growth, and gingerol content

Neena Adichirattle*, Purathatt Mahesh Mohanan, Binu Thomas

Centre for Post Graduate Studies and Research in Botany, St. Joseph's College (Autonomous), Devagiri, Calicut-673 008, Kerala, India

Abstract

Globally, environmental stress plays a significant role in the growth and development of plants. Every stress alters the signal system, eventually resulting in an end response. Combination of stress treatments can both negatively or positively affect the plant. A few studies have been conducted on combined stress treatments in ginger. In the present study, the effect of various individual stress treatments and their combined treatments on the concentrations of gingerols were analyzed in the improved ginger variety (*Zingiber officinale* cv. varada). The different stress treatments were attempted using salicylic acid and zinc sulphate. Along with these two, drought was also taken as a third stress. The results of individual and combination of stress treatments were obtained. The study presented insights on the effect of individual as well as combined stress treatments on both morphological (yield and growth parameters) and the gingerol content. The study also provides for a clear picture of cross tolerance in which one stress influences the effect of another.

Keywords: cross tolerance, drought, gingerol, salicylic acid, zinc sulphate

Adichirattle, N., P. Mahesh Mohanan, and B Thomas. 2022. 'Differential response of *Zingiber officinale Rosc.* Cv. varada to solitary and combined stress treatments with respect to yield, growth, and gingerol content'. *Iranian Journal of Plant Physiology 12*(4), 4281-4287.

Introduction

Environmental stresses have a significant negative impact on current agriculture. Under field conditions, commercially grown crops achieve an average of only about 50% of their potential yield due to the negative effects of abiotic environmental stresses such as drought, poor soil quality, temperature extremes, and flooding (Hatfield and Walthall, 2015).

Plants are able to withstand multiple mild and even severe environmental stresses simultaneously (Atkinson and Urwin, 2012). In a competitive growing environment a key factor underlying such resistance is the capacity of sessile plants to recognize, integrate, and respond to biotic and abiotic environmental variables by constantly adjusting physiology and metabolism to optimize growth and reproduction in an ever-changing environment. This capacity is facilitated by cross-tolerance phenomena, in which enhanced tolerance to a range of different environmental stresses is triggered by exposure to a single stress (Foyer et al., 2016); Mittler, 2006). Combination of stresses can mask the effect of individual stresses thereby increasing the plant productivity in terms of quantity as well as quality (Foyer et al., 2016).

* *Corresponding Author*
E-mail Address: ninaprabhakar@gmail.com
Received: November, 2021
Accepted: Februry, 2022

Ginger (*Z. officinale* Rosc.) is an herbaceous, perennial plant of about 1 m in height with many fibrous roots, aerial shoot with leaves, and branched rhizome. The rhizomes are thick lobbed, branched with structures with spicy scent (Ravindran et al., 2016). It is one of the most important and ancient spice crops in India. It belongs to the family *Zingiberaceae* and a tropical and sub-tropical perennial herb with underground branching stem called rhizomes. It is native to South East Asia. India is the largest producer in the world. Ginger plays an important role in earning foreign exchange for India (Babu et al., 2017)with an annual production of 6.55 L tones in an area of about 1.33L hectares comprising approximately 65% of the world production. The share of ginger production among the spices in India is 11.89% (NHB Database 2014-15). Ginger is grown as an intercrop in coconut and arecanut plantations in the states of Kerala, Meghalaya, Orissa, West Bengal, and to some extent in Karnataka as well as pure crop in states of Andhra Pradesh, Tamil Nadu. Its cultivation is fast increasing as a pure crop particularly in these states because of better profitability and high productivity (Gaikwad et al., 2017). It requires a well distributed rainfall during growing season and hence drought stress early during the growth phase is a menace (Vivek et al., 2013).

Very limited information is available on the varietal evolution of ginger in terms of gingerol content and morphological parameters in response to stresses especially to foliar sprays and its combinations. If any foliar spray of chemical compound can alter the negative impact of drought stress in ginger it can be a breakthrough in the improvement of this commercial crop. To date, much less focus has been placed on the integrated response of plants to multiple stresses typically encountered under field conditions; however, fundamental knowledge is now sufficiently advanced to tackle these questions. In the present study an attempt was made to assess the impact of solitary stress and comparing it with combined stresses for morphological parameters and total gingerol content.

Materials and Methods

Plant material, maintenance, and stress treatments

The study was conducted in a rain-protected green house. *Zingiber officinale* Cv. *varada* was used for the present study. Ginger seed rhizomes were collected from Indian Institute of Spices Research, Kozhikode (ICAR Unit- IISR Kozhikode) and maintained in the greenhouse of St. Joseph's College (Autonomous) Devagiri, Kozhikode, Kerala at 28 ℃. Rhizomes were grown in growth bags containing soil and coco peat in the ratio of 2:1. There were about 15 growth bags kept for every single stress treatment. When the ginger seedlings were at the second leaf stage (approximately 90 days after planting), they were sprayed with two concentrations (10^{-2} and 10^{-3} M) of salicylic acid solution (SA; 2-hydroxybenzoic acid + 100 µl dimethyl sulfoxide + 0.02% Polyoxyethylenesorbitan monolaurate, Tween 20, Sigma Chemicals; pH 6.5) and zinc sulphate with the same molar concentrations ($ZnSO_4.7H_2O$ dissolved in distilled water + 0.02% Polyoxyethylenesorbitan monolaurate, Tween 20, Sigma Chemicals; pH 4). Control plants were sprayed with the same solution but without SA and $ZnSO_4$. A trial was also maintained without any foliar application to estimate the amount of non-volatile components of the variety under study. Apart from foliar spray, drought stress was imposed by withholding water application at the trials. Plants were sprayed once on the leaves early in the morning and every week until one month.

Analysis of morphological data

Five plants were selected at random and studied for growth characters. Performance of ginger varieties for growth attributes like plant height, number of leaves per plant, and number of tillers per plant were evaluated after 180 days of planting and yield attributes like weight of fresh rhizomes were measured at the end of the harvest, and the data was recorded.

Sample preparation and extraction

Harvested rhizomes were shade-dried and weighed to monitor their dry weight. They were then powdered separately and from each sample 1 g sample powder was taken and submitted to cold extraction in methanol. Samples were dissolved in 100 ml methanol in a standard flask, shaken for 2 hours, and kept overnight. From surface of the sample 20 ml of the supernatant was pipetted to a round bottom flask and evaporated to 2 ml in a rotary evaporator (Buchi Rotavapor B-100). This extract was then made up to 5 ml with HPLC methanol in a 5 ml standard flask, shaken well, and filtered using a syringe filter.

Preparation of standard and instrumentation

Gingerol reference standard was prepared by dissolving 0.1 g of 6-gingerol in 100 ml HPLC methanol in a 100 ml standard flask. From this reference standard, 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, and 0.8 mg/ml were prepared. Standard curve was plotted for these concentration using HPLC. The general fingerprints were obtained by HPLC Photo Diode Array (PDA) detector using Shimadzu C10- 10 AS. The mobile phase consisted of Acetonitrile and HPLC Water (65;35) in 1% acetic acid, filtered, and sonicated for 30 minutes and used. The flow rate was 1 ml/minute. The operating temperature was maintained at 23 ℃.

Results

Single variety of ginger (*Zingiber officinale* cv-varada) under various solitary as well as combined stress treatments were evaluated for variation in morphological as well as in non-volatile components. Morphological parameters under study included plant height, number of leaves per plant, number of tillers per plant, and yield attributes characterized by fresh and dry weight of the rhizome. Concentrations of non-volatile components such as 6- gingerol were also monitored under different stress treatments and were compared with controls. A comparative study was made on morphological parameters with concentration of gingerol content. It was then compared with standard chromatogram of the gingerol and standard curve (Figs. I and II, respectively).

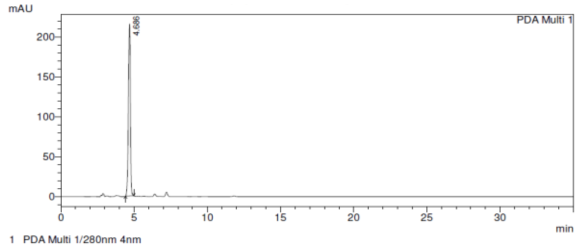

Fig. I. Chromatogram of 6-gingerol 0.1mg/ml

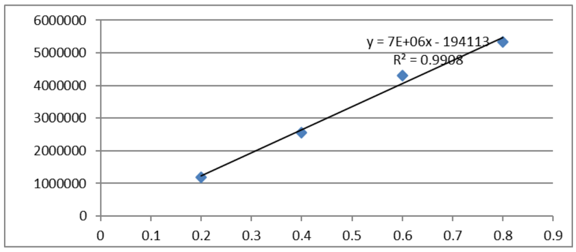

Fig. II. Standard curve for 6- Gingerol

A reverse trend was observed while comparing morphological parameters with the gingerol concentration. Plant which responded positively in terms of morphological parameters showed a negative trend towards the gingerol concentration and vice-versa. Morphological parameters and yield attributes are presented in Tables 1 and 2, respectively.

Effect of salicylic acid on morphological parameters and gingerol content

Vegetative growth was found to be more prominent in plants sprayed with salicylic acid compared with the other treatments. Spraying with 10^{-2} molar salicylic acid showed significant effect on the number of leaves, height of the plants, and fresh and dry weights of the rhizome as compared with 10^{-3} molar salicylic acid treatment. At the same time gingerol content was found to be less comparing with the plants treated with foliar spray of 10^{-3} concentration of salicylic acid.

Another interesting finding concerning with salicylic acid foliar spray was that its control showed high gingerol content compared with any other concentrations of foliar spray. Since salicylic acid is non-polar, its foliar spray was prepared by dissolving the salicylic acid on Dimethyl sulfoxide

Table 1
Performance of ginger (*Zingiber officinale* cv. *varada*) Variety: Varada for growth attributes (after 180 days of planting)

Treatments	Height of the plant(cm)	No. of leaves per plant	No. of tillers per plant
SA (Control with DMSO)	80	13.22	1
SA 10^{-2} Molar	90.2	14.33	3.5
SA 10^{-3} Molar	84.2	14.1	2
SA 10^{-2}Molar+ drought	86.3	13	2
SA 10^{-3} Molar+ drought	85	13.21	1
Drought (C)	70.3	10.1	1
$ZnSo_4$ (C)	72.8	10.7	2
$ZnSo_4$ 10^{-2}Molar	73.7	10.8	1
$ZnSo_4$ 10^{-3} Molar	76.8	11	1
$ZnSo_4$ 10^{-2}Molar+ Drought	77	12	1
$ZnSo_4$ 10^{-3} Molar+ Drought	78	13.5	2
SA 10^{-3} Molar +$ZnSo_4$ 10^{-3} Molar	75	12.8	1
SA 10^{-2}Molar +$ZnSo_4$ 10^{-2}Molar	85	13.5	1
SA 10^{-3} Molar+ $ZnSo_4$ 10^{-2} Molar	84	12.9	1
SA 10^{-2} Molar+ $ZnSo_4$ 10^{-3} Molar	82	11.2	1

Table 2
Performance of ginger (*Zingiber officinale* cv. varada) for yield attributes (after 180 days of planting)

Treatments	Rhizome fresh weight	Rhizome dry weight
SA (Control with DMSO)	285	49.02g
SA 10^{-2}Molar	435g	60.484g
SA 10^{-3} Molar	400g	55.9g
SA 10^{-2}Molar+ Drought	305g	51g
SA 10^{-3} Molar+ Drought	280g	48.7g
Drought (C)	260 g	42.19g
$ZnSo_4$ (C)	275g	44.15g
$ZnSo_4$ 10^{-2}Molar	355g	52g
$ZnSo_4$ 10^{-3} Molar	275g	47g
$ZnSo_4$ 10^{-2}Molar+ Drought	210 g	40.4g
$ZnSo_4$ 10^{-3} Molar+ Drought	270g	42.1g
SA 10^{-3} Molar +$ZnSo_4$ 10^{-3} Molar	245g	43g
SA 10^{-2}Molar +$ZnSo_4$ 10^{-2}Molar	260g	47g
SA 10^{-3} Molar+ $ZnSo_4$ 10^{-2} Molar	256g	45.2g
SA 10^{-2} Molar+ $ZnSo_4$ 10^{-3} Molar	258g	45g

(DMSO). In other words, controls were sprayed with the solution without salicylic acid but with DMSO. To monitor the effect of DMSO on plant characteristics under study, a separate control was considered in the study where plants were sprayed with only distilled water. The study showed that DMSO had a positive effect on gingerol content and a negative effect on vegetative/morphological parameters. (Fig. III)

Effect of combination application of salicylic acid and drought stress on morphological parameters and gingerol content

Decreasing field capacity and increasing SA concentration had a significant role in increasing gingerol content as well as the morphological parameters such as plant height, number of leaves, and number of tillers per plant comparing with the control plants (100% field capacity irrigation with distilled water) as well as with solitary spray of salicylic acid (Fig. III).

Effects of zinc sulphate on morphological parameters and gingerol content

Spraying with 10^{-2} and 10^{-3} molar concentrations of zinc sulphate solution showed that increasing zinc sulphate concentration to 10^{-2} M had a significant positive effect on gingerol concentration and a negative impact on morphological parameters like stem height, number of leaves, and fresh and dry weights of the rhizome (Fig. IV).

Fig. III. The effect of foliar spray of SA: Solitary application as well as in combination with drought stress (here control is with DMSO)

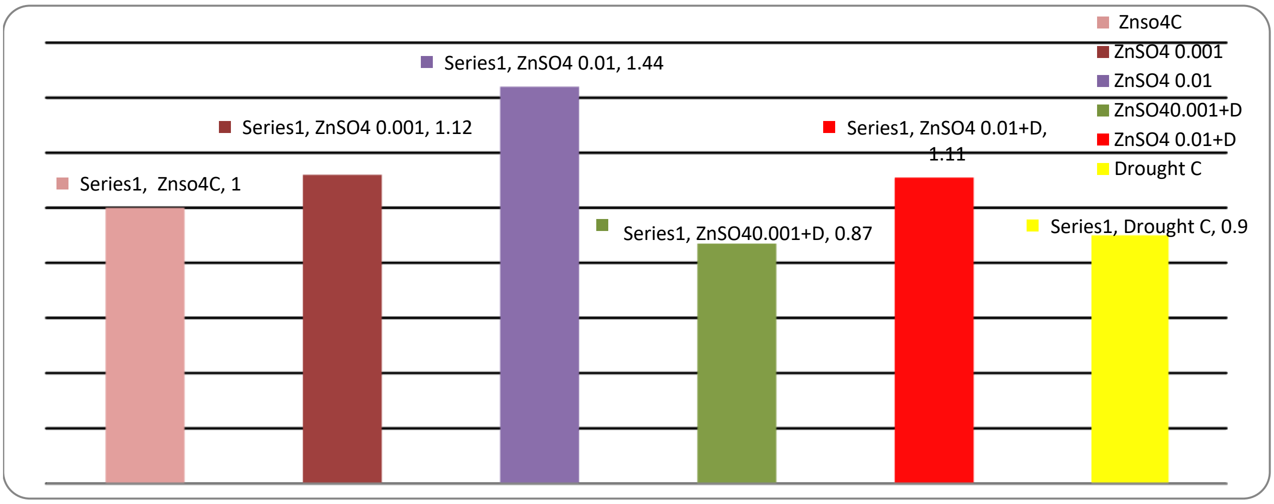

Fig. IV. The effects of foliar spray of $ZnSo_4$ solitary application as well as in combination with drought stress (control is sprayed with distilled water.)

Combined effect of zinc sulphate and drought stress on morphological parameters and gingerol content

Combination of zinc sulphate foliar spray and drought stress showed a negative impact on gingerol content and morphological parameters. Foliar spray along with drought stress reduced the gingerol concentration as well as the vegetative growth of the plant. It was observed that the effect of one solitary foliar spray reversed under influence of another foliar spray. That is, the effect of solitary foliar spray of zinc sulphate (10^{-3} M) was reversed by SA at the same concentration and vice-versa while morphological parameters did not show any considerable variation (Fig. IV).

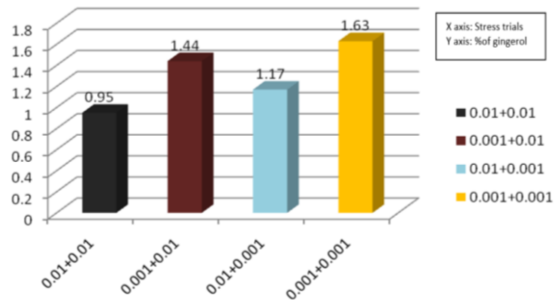

Fig. V. Combined effect of foliar sprays of SA and $ZnSo_4$

Combined effect of SA and zinc sulphate on morphological parameters and gingerol concentration

High concentration of both zinc sulphate and SA (10^{-2} molar) significantly reduced the gingerol content while lower concentration (10^{-3} molar) of zinc sulphate and SA had a positive effect on gingerol content. Next to this, 10^{-2} molar SA with

10^{-3} molar zinc sulphate and then a combination of 10^{-3} molar SA with 10^{-2} molar zinc sulphate showed high gingerol content. (Fig. V).

Discussion

Increasing concentration of salicylic acid had a positive effect on morphological parameters and at the same time it had a negative effect on gingerol content. In a similar study (Ghasemzadeh and Jaafar, 2012) investigated the foliar treatments of salicylic acid for their biochemical properties in ginger. Their results showed that foliar spray of salicylic acid greatly increased the synthesis of phenolic acids in ginger leaves.

Furthermore, the study suggested that foliar spray of salicylic acid has a positive effect on vegetative growth of the plant as compared with the foliar spray of zinc sulphate. Spraying zinc sulphate had a positive impact on concentration of gingerol content. Considering gingerol content, lower concentration of SA (10^{-3} M) resulted in comparatively high amount of gingerol than 10^{-2} M. A reverse trend was observed in foliar spray of zinc sulphate. In combination with drought stress, salicylic acid showed a positive cross tolerance. Similar studies were conducted by Khalil et al. (Khalil et al., 2018)on *Thymus vulgaris* where the negative impact of drought was reversed by the foliar application of salicylic acid. Thus, it can be inferred that the combination of two stresses had a positive impact on the parameters under study. On the other hand, zinc sulphate showed a negative cross tolerance and its interaction with SA had a negative impact on the parameters under study. In fact, a cumulative effect can be observed in applying the two stresses to the trial plants. Results of the combined treatment of salicylic acid and zinc sulphate indicated the phenomenon of cross tolerance in which one stress masked the effect of another. In the control plants for salicylic acid where DMSO was applied, the study also suggested a high amount of gingerol content and a negative effect on vegetative parameters. Thus, it can be inferred that DMSO has a positive effect on gingerol content and a negative effect on vegetative growth.

Acknowledgement

I gratefully acknowledge the funding agency, the University Grant Commission (*UGC*) of the Government of India, for providing financial support, in the form of UGC-CSIR NET-JRF/SRF.

References

Atkinson, N. J. and P. E. Urwin. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of experimental botany,* 63, (10) 3523-3543.

Babu, M. S., B. P. Kumar, D. Swami, K. U. Krishna and N. Emmanuel. 2017. Performance of ginger (Zingiber officinale Rosc) varieties under shade net condition of costal Andhra Pradesh. *International Journal of Current Microbiology and Applied Sciences,* 6, (7) 494-498.

Foyer, C. H., B. Rasool, J. W. Davey and R. D. Hancock. 2016. Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation. *Journal of experimental botany,* 67, (7) 2025-2037.

Gaikwad, D., S. K. Shinde, A. V. Kawade, S. Jadhav and M. Gadhave. 2017. Isolation and standardization of gingerol from ginger rhizome by using TLC, HPLC, and identification tests. *The Pharma Innovation,* 6, (2, Part C) 179.

Ghasemzadeh, A. and H. Z. Jaafar. 2012. Effect of salicylic acid application on biochemical changes in ginger (Zingiber officinale Roscoe). *Journal of Medicinal Plants Research,* 6, (5) 790-795.

Hatfield, J. L. and C. L. Walthall. 2015. Meeting global food needs: realizing the potential via genetics× environment× management interactions. *Agronomy Journal,* 107, (4) 1215-1226.

Khalil, N., M. Fekry, M. Bishr, S. El-Zalabani and O. Salama. 2018. Foliar spraying of salicylic acid induced accumulation of phenolics, increased radical scavenging activity and modified the composition of the essential oil of water stressed Thymus vulgaris L. *Plant Physiology and Biochemistry,* 123, 65-74.

Ravindran, P., K. N. Babu and K. Shiva. 2016. Botany and crop improvement of ginger. In *Ginger*:35-106: CRC Press. Number of 35-106 pp.

Vivek, P. J., N. Tuteja and E. V. Soniya. 2013. CDPK1 from ginger promotes salinity and drought stress tolerance without yield penalty by improving growth and photosynthesis in Nicotiana tabacum. *PLoS One*, 8, (10) e76392.