International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(1) January -2016

METHYL-JASMONATE INDUCED ACCUMULATION OF VITAMINS AND OSMOLYTES IN *CAJANUSCAJ AN* (L.) MILLSP.UNDER COPPER STRESS

Geetika Sirhindi, Poonam Sharma., Harpreet Kaur and Mudaser Ahmad Mir

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 1, pp. 8451-8454, January, 2016

International Journal of Recent Scientific Research

ISSN: 0976-3031

RESEARCH ARTICLE

METHYL-JASMONATE INDUCED ACCUMULATION OF VITAMINS AND OSMOLYTES IN *CAJANUSCAJ AN* (L.) MILLSP.UNDER COPPER STRESS

Geetika Sirhindi, Poonam Sharma*., Harpreet Kaur and Mudaser Ahmad Mir

Department of Botany, Punjabi University, Patiala-147 002, Punjab, India

ARTICLE INFO

Article History:

Received 15th October, 2015
Received in revised form 21st November, 2015
Accepted 06th December, 2015
Published online 28st January, 2016

Key words:

Methyl jasmonate, copper, vitamins, oxidative stress, osmolytes for vitamin B2 add 2 as subscript

ABSTRACT

An experiment was conducted to study the effect of methyl jasmonate (Me-JA) seed priming on osmolytes and vitamins in 15 days old seedlings of *C. cajan* under copper stress. Me-JA treated *C. cajan* seedlings were grown in petriplates lined with Whatman filter paper containing different concentration of copper sulphate (Cu) in seed germinator for 15 days. After 15 days proline, glycine betaine, vitamin A, vitamin B_2, vitamin C and vitamin E were studied. Proline accumulation was increased to 430% in seedlings grown in 5mm Cu with Me-JA treatment and increased to 514.2% in 1µM Me-JA treated seedlings grown in 1mM Cu stress. Similarly glycine betaine content was increased to 103.4% in 1pM Me-JA treated seedlings grown in 5mM Cu stress. Vitamin A content was increased to 44.01% in 10mM Cu as compared to the control which was only 20.3% in 1µM Me-JA treated seedlings grown in 10mM Cu stress. Vitamin B^2 was however decreased in all the treatments. Vitamin C content was also increased to 224% in 1pM Me-JA treated seedlings grown in 10mM Cu stress. it was concluded that Me-JA help plant in adjusting to Cu stress condition by increasing proline , glycine betaine and vitamin content in *C. cajan* seedlings.

INTRODUCTION

Pigeonpea [*Cajanuscaj an* (L.) Millsp.]is most widely grown legume crop in the world. The Indian subcontinent account for about 92% of its production worldwide. Pigeonpea due to its wide ranging production may face different type of biotic and abiotic stress. Copper (Cu) is one of the micronutrient which can be a stress factor in deficiency as well as in excess in soil. Copper is an important metal ion needed for growth and development and is a part of pigments as well as enzymes such as superoxide dismutase, polyphenol oxidase, tyrosinase (Yurela2005).Mohammadi *et al*. (2013) reported increase in seed germination percentage in *Plantagopsyllium* seeds. However, Cu stress leads to decrease in seed germination, root length and shoot length (Ashagre *et al.*, 2013). Decline in shoot length and root length occur due to decline in α-amylase, acid phosphatase and alkaline phosphates activities. Cu stress also leads to decrease in photosynthetic pigments, total protein content (Guleryuz *et al.*, 2015) and increasein proline content, total soluble sugars. (Poonam *et al.*, 2013; Sirhindi *et al.*, 2015). Ritter *et al*. (2008) reported that Cu stress induces octadecanoid and eicosanoid oxygenated derivatives in the brown algal kelp *Laminariadigitata*. Wang *et al*. (2015) reported that allene oxide cyclase gene GhAOC1 which is an essential enzyme in JA biosynthetic pathway in *Gossypiumhirsutum* L. ameliorateCu stress tolerance in Arabidopsis by transgenic expression.

Jasmonic acid, its methyl ester methyl- jasmonate (Me-JA) collectively called Jasmonates are oxylipins molecules which are synthesized from fatty acid by octadecanoid pathway during biotic and abiotic stresses. JAs play important role in seed germination, root formation, trichome formation, tendril coiling, senescence and stress protection. Exogenous application of JAs may help in protecting plants from various types of stresses such as drought, temperature, light, herbivory by altering various physiological parameters. Linkies and Leubner-Metzger (2012) reported the role of Me-JA on germination and seedling growth. The inhibitory effect of jasmonates on seed germination in maize may result due to decrease in concentration and activity of α-amylase and reduction in ethylene production. Exogenous application of Jasmonates (Me-JA or JA) leads to increase in total chlorophyll in *Brassicanapus* (Kaur *et al.*,2013), Sweet basil (Sorial*et al.*,2010), tulip bulb (Sanewski *et al.*, 2006) and chlorophyll a and chlorophyll b content (Jamalmidi *et al.*, 2013). JA application also increases total proteins (Kumari *et al.* 2006), proline content (Karimi *et al.*, 2012) and glycine betaine content (Sirhindi *et al.*, 2015). Gao *et al*. (2004) reported that JA application leads to accumulation of glycine betaine under drought stress in *Pyrusbretschneideri* due to upregulation of

Corresponding author:* **Poonam Sharma
Department of Botany, Punjabi University, Patiala-147 002, Punjab, India

betaine aldehyde dehydrogenase gene expression. Wolucka *et al*. (2005) disclosed that Me-JA treatment leads to increased synthesis of ascorbic acid in Arabidopsis and tobacco bright yellow 2 (BY-2) suspension cells. Similarly total soluble sugars, vitamin C content of tomato fruit was significantly affected by SA and Me-JA which contributed to the significant increase in tomato fruit yield (Kazemi, 2014). The present study was undertaken to analyse the magnitude of the shielding of Me-JA seed priming in *C. cajan* seedlings under Cu stress in terms of osmolytes and antioxidants accumulation.

MATERIAL AND METHODS

Plant material and stress treatments

Cajanuscajan AL 201 seeds were brought from Department of Plant Breeding and Genetics, Punjab Agriculture University, Ludhiana, India. Healthy seeds were washed with water and with 5% (w/v) tepol for 10 minutes and then sterilized with 5% hypochloride (v/v). Then surface sterilized seeds were immersed in different concentrations of Me-JA and DW as control, for 6 hours. These treated and non-treated seeds were grown in petriplates lined with Whatman No.1 filter paper and having 10mM of different concentrations (0, 1mM, 5mM and 10mM) Cu sulphate concentrations. These seed were grown in petriplates in seed germinator at 24˚C under controlled condition (200 PAR light, 80% humidity and 16±8 photoperiod).15 days after sowing (DAS) seedlings were harvested and used for various biochemical analyses. Our experiment consisted of 16 treatments with 3 replication of each treatment. The treatment included control (DW), 1mM, 5mM and 10mM Copper sulphate, 1µM, 1nM and 1pMMe-JA and combination of various concentrations of Me-JA and Cu.

Proline content

Proline was evaluatedusing Bates *et al*.(1973) method. Plant tissue was homogenized in 3% sulphosalicylic acid and centrifuged at 11,500X g. The homogenate was filtered followed by addition of glacial acetic acid and acid-ninhydrin. Sample was incubated at 100 °C for 1 h toluene was added and absorbance was taken at 520 nm.

Glycine betaine

Plant tissue was mechanically shaken with 20 ml of deionized water. 2 N sulphuric acid was added to thawed extracts in 1:1 ratio and added cold potassium iodide-iodine reagent. Stored the reaction mixture at 0-4^{o}C for 16 h. The supernatant was removed carefully after centrifugation and the periodite crystals were dissolved in 9 ml of 1,2-dichloro ethane (reagent grade). Absorbance was measured at 365 nm.

Vitamin A

Vitamin A estimation was done according to Bayfield and Cole (1980) method. Homogenate was mixed with 1.0ml of saponification mixture and refluxed at 60°C in the dark followed by cooling with 20ml water. Vitamin A was extracted with 10ml petroleum ether. TCA reagent (2.0ml) was added rapidly with absorbance at 620nm in a spectrophotometer.

Vitamin B$_2$

Vitamin B$_2$ estimation was done according to I. P (1996) method. Reaction mixture contained plant tissue powder, water and glacial acetic acid. The solution was boiled for 5 minutes followed by cooling. After that, 30 ml of 1.0 M sodium hydroxide solution was added and diluted to 550 ml with water. The solution was then filtered and absorbance was recorded at 444 nm in Shimadzu UV-1201 spectrophotometer.

Vitamin C

Vitamin C was estimated according to the Chinoy*et al*.. (1976) method. 2 ml of plant extract was prepared and added 8 ml of 2, 6-dichlorophenol indophenols dye. OD was recorded at 530 nm.

Vitamin E

VitaminE was estimated by using Rosenberg (1992) method. Sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight and then filtered. To the 1.5 ml of tissue extract added 1.5ml of xylene and centrifuged. Then 1.0ml of xylene was separated and mixed with 1.0ml of 2, 2-pyridyl and noted the absorbance at 460nm. Then in the beginning with blank added 0.33 ml $FeCl_3$ and mixed well. After 15 minutes read the test and standard against the blank at 520nm.

RESULTS

Proline accumulation (Figure 1) in Me-JA treated plants of *Cajanuscajan* was increased and highest increase was seen in 1pM Me-JA which was upto 252%.

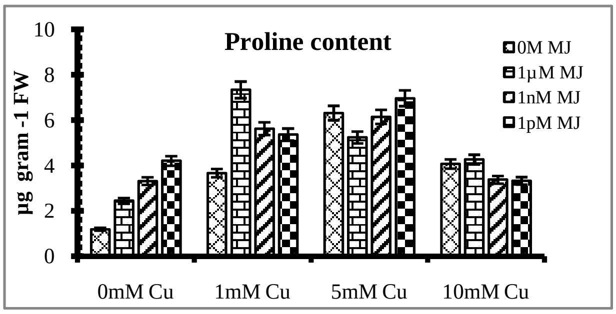

Figure1 Showing the effect of Me-JA treatment on proline content in Cu stressed seedlings of *C. cajan*

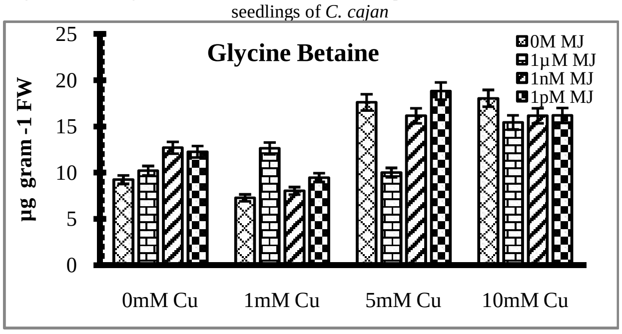

Figure 2 Showing the effect of Me-JA treatment on glycine betainecontent in Cu stressed seedlings of ***C. cajan***

Similar increase in proline content in response to Me-JA was reported in seedlings treated with 1µM Me-JA and grown in

5mM Cu stress and upto 430% increase was recoeded. Glycine betaine was decreased in 1mM Cu to 21.2% and increased in 5mM and 10mM Cu treatments to 90.13% and 94.9 % respectively (Figure 2). Me-JA treatment also increased glycine betaine content and highest increase of 37.19% was seen in 1nM Me-JA treated seedlings grown in distilled water and 103.4% in 1pM Me-JA treated seedlings grown in 5mM Cu stress. Me-JA as well as Cu also increased Vitamin A content in *C. cajan* seedlings (Figure 3).

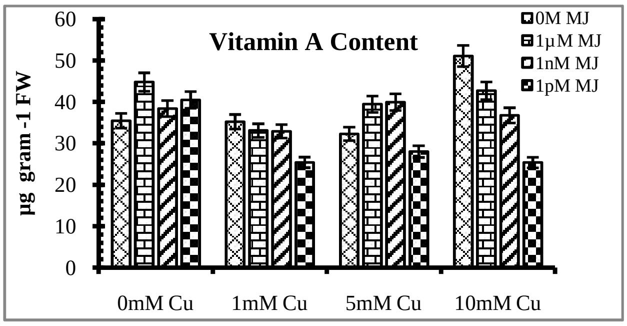

Figure 3 Showing the effect of Me-JA treatment on vitamin A content in Cu stressed seedlingsof *C. cajan*

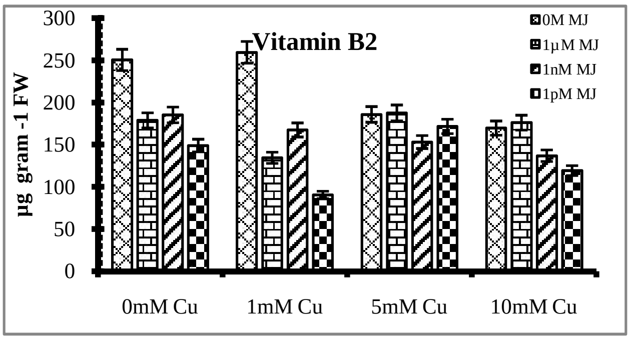

Figure 4 Showing the effect of Me-JA treatment on vitamin B_2 content in Cu stressed seedlings of *C. cajan*

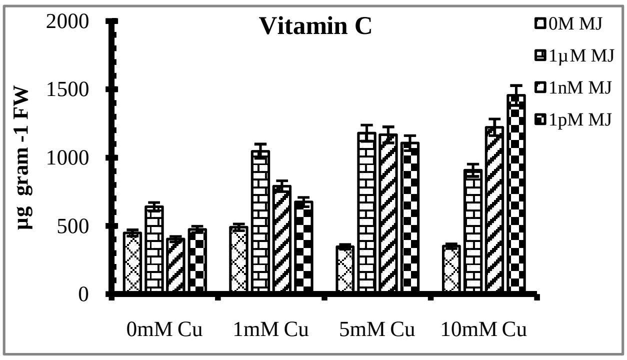

Figure 5 Showing the effect of Me-JA treatment on vitamin C content in Cu stressed seedlingsof *C. cajan*

However, decrease in vitamin B_2 content was observed in different concentration of Me-JA as well as Cu (Figure 4). Highest increase of 26.2% in vitamin A content was reported in 1µM Me-JA treated seedlings grown in distilled water Even though in Cu stress, highest increase upto 44.0 % was seen in case of seedlings grown in 10mM Cu treatment. Vitamin C content was also increased in different concentrations of Me-JA and Cu and highest increase of 9.38% was seen in 1mM Cu stress (Figure 5). 1µM Me-JA showed upto 42% increase and 224% increase was observed in 1pM Me-JA treated seedlings grown in 10mM stress. Vitamin E content was also increased in Cu stress treatment and highest increase was upto 147% (Figure 6). In Me-JA treatment vitamin E content was increased upto 663% and highest increase of 243% was seen in seeds treated with 1µM Me-JA grown in Cu stress conditions.

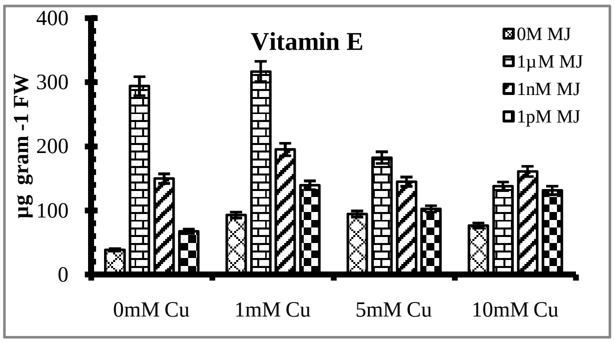

Figure 6 Showing the effect of Me-JA treatment on vitamin E content in Cu stressed seedlings of *C. cajan*

DISCUSSION

Cajanuscajan seedlings when grown in different concentration of Cu stress face osmotic as well oxidative stress. To regulate this osmotic stress plant produces osmoprotectants such proline, glycine beatine, sarcosine, myinositol. In the present study increase in proline content as well as glycine beatine content was recorded in Cu stress as well as Me-JA treatments. Mohammadi *et al.* (2013) also conveyed similar increase in Proline content in $CuSO_4$ treatment in Psyllium seedlings.

Similar increase in proline content in *Solanumnigrum* was reported by Al Khateeb and Al-Qwasemeh (2014). Karimi *et al.* (2012) also documented increase in proline content in *Astragalus neo-mobayenii*. Gao *et al.* (2014) also reported similar increase in glycine betaine content in *Pyrusbretschneideri*. This increase in glycine betaine is causeddue to up regulation of betaine aldehyde dehydrogenase. Vitamins such as vitamin A, vitamin B_2, vitamin C, vitamin E act as non-enzymatic antioxidants in the plants and protect them from environmental stress by scavenging reactive oxygen species formed under stress condition.

Vitamin A content and Vitamin C content is also increased in Me-JA as well as Cu in *C. cajan* seedlings. However decrease in vitamin B_2 content was observed in different concentration of Me-JA as well as Cu. Sirhindi *et al.* (2015) also reported increase in vitamin A content in JA treated *C.cajan* seedlings. Similarly, Wolucka *et al.* (2005) reported increase in ascorbic acid synthesis in Me-JA in arabidopsis and tobacco bright yellow (BY-2) suspension cells. Vitamin E content was also increased in Cu stress treatment.

CONCLUSION

It can be concluded that maintenance of osmoregulation is an important mechanism for increasing oxidative stress tolerance. Me-JA is showing its important role increasing level of various osmolytes and antioxidants. Higher level of proline and glycine betaine played an important role in maintaining better osmoregulation in *C. cajan* seedlings whereas vitamins help in oxidative stress maintenance under Cu stress condition.

Acknowledgement

Authors highly acknowledge the Department of Science and Technology (DST) New Delhi for financial support in the form of WOS-A project.

References

1. Al Khateeb, W. and Al-Qwasemeh, H. 2014. Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of *Solanumnigrum* L., a crop wild relative for tomato; comparative study. *PhysiolMolBiol Plants.* 20(1):31-9.
2. Ashagre, H., D., Almaw, and Feyisa, T. 2013. Effect of copper and zinc on seed germination, phytotoxicity, tolerance and seedling vigor of tomato (*Lycopersiconesculentum* L. cultivar Roma VF). *Int J Agric Sci.* 2(11) 312-317
3. Bates, L. S., R. P. Waldren and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* 39:205-208
4. Bayfield, R. F. and Cole, E. R. 1980. Colorimetric determination of vitamin A with trichloroacetic acid. In: McCormick, D.B. and Wright, L.D., Eds. Methods in Enzymology, part F. Vitamins and Coenzymes. 67. New York: Academic Press. 189-195
5. Chinoy, J. J., Y.D. Singh and Gurumurthi, K. 1976. The role of ascorbic acid in growth, differentiation and metabolism of plants. *J Plant Physiol.* 22: 122
6. Gao, X.P., X.F. Wang, Y.F., Lu, L.Y. Zhang, Y.Y., Shen, Z. Liang and Zhang D.P. 2004 Jasmonic acid is involved in the water-stress-induced betaine accumulation in pear leaves. *Plant Cell Environ*. 27(4):497 - 507
7. Greive, C. M. and Grattan, S. R. 1983. Rapid assay for determination of water-soluble quaternary amino compounds. *Plant Soil.* 70: 303-307
8. I.P., 1996. Indian Pharmacopeia. Addendun, 2000. The controller of publications, Govt. of India, ministry of Health and family welfare, Delhi, India.
9. Karimi, P., R., Khavari-Nejad,V.,Niknam, F. Ghahremaninejad,andNajafi, F. 2012. The effects of excess copper on antioxidative enzymes, lipid peroxidation, proline, chlorophyll, and concentration of Mn, Fe, and Cu in *Astragalus neo-mobayenii*. *Sci World J*. 2012:1–6.
10. Kazemi, M. 2014. Effect of foliar application with salicylic acid and methyl jasmonate on growth, flowering, yield and fruit quality of tomato. *Bull EnvPharmacol Life Sci.* 3 (2) :154-158
11. Kaur, H., P. Sharma and Sirhindi, G. 2013. Sugar accumulation and its regulation by jasmonic acid in *Brassica napus* L. under salt stress. *J Stress PhysiolBiochem.* 9:53-64.
12. Kumari, G.J., A.M., Reddy, S.T. Naik, S.G. Kumar, Prasanthi, G., Sriranganayakulu, P.C., Reddy and Sudhakar, C. 2006. Jasmonic acid induced changes in protein pattern, antioxidative enzyme activities and peroxidase isozymes in peanut seedlings. *Biol Plant.* 50:219–226
13. Linkies, A. and Leubner-Metzger, G. 2012. Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. *Plant Cell Rep* 31:253–270
14. Mohammadi, Z., S. M. N. Kalat, and Haghaghi, R. S. 2013. Effect of copper sulfate and salt stress on seed germination and proline content of Psyllium(*Plantagopsyllium*). *American-Eurasian J Agric Environ Sci.*13(2):148–152.
15. Poonam, S., K. Harpreet, Geetika, S. 2013. Effect of jasmonic acid on photosynthetic pigments and stress markers in *Cajanuscajan* (L.) Millsp. seedlings under Cu stress. *Amer J Plant Sci.* 4:817-823.
16. Ritter, A., S. J.P., Goulitquer, T. Salaün, J. A, Tonon, Correa and Potin, P. 2008. Copper stress induces biosynthesis of octadecanoid and eicosanoid oxygenated derivatives in the brown algal kelp *Laminariadigitata*. *New Phytol* 180(4): 809–21.
17. Rosenberg, H.R. 1992. Chemistry and physiology of vitamins. Inter Science Publishers Inc., New York. 452–3.
18. Saniewski, A., M., Horbowicz, and Puchalski, J. 2006. Induction of anthocyanins accumulation by methyl jasmonate in shoots of *Crassulamulticava*Lam. *ActaAgrobotanica*59: 43–50.
19. Sirhindi, G., P. Sharma, A. Singh, H. Kaur, and Mir, M. 2015. Alteration in photosynthetic pigments, osmolytes and antioxidants in imparting copper stress tolerance by exogenous jasmonic acid treatment in *Cajanuscajan. Int J Plant PhysiolBiochem*7:30-39.
20. Sorial, M.E., S.M., El-Gamal and Gendy, A.A. 2010. Response of sweet basil to jasmonic acid application in relation to different water supplies. *Biosci Res* 7:39–47.
21. Wang, Y., H. Liu, and Q. Xin, 2015. Improvement of copper tolerance of Arabidopsis by transgenic expression of an allene oxide cyclase gene, GhAOC1, in upland cotton (*Gossypiumhirsutum* L.). *Crop J*. 3:(4) 343–352.
22. Wolucka, B. A., Goossens, A. and Inzé, D. 2005. Methyl jasmonate stimulates the *de novo* biosynthesis of vitamin C in plant cell suspensions. *J Exp Bot.* 56: 2527–2538.
23. Yruela, I. 2005. Copper in plants. Braz J Plant Physiol. 17:145-156

****

How to cite this article:

Geetika Sirhindi *et al*.2016, Methyl-Jasmonate Induced Accumulation of Vitamins and Osmolytes in Cajanuscaj an (L.) Millsp.Under Copper Stress. *Int J Recent Sci Res.* 7(1), pp. 8451-8454.

ISSN 0976-3031
9 770976 303009 >