Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (4):490-495

ISSN : 2249-7412
CODEN (USA): AJPSKY

Laminarin as a potential non-conventional elicitor for enhancement of capsaicinoid metabolites

Harishchandra B Gururaj, Parvatam Giridhar and Gokare A Ravishankar*

Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute, Mysore-570020, India

---

ABSTRACT

Capsaicinoids are alkaloids responsible for the pungency factor in the fruits of Capsicum sp. Capsaicin finds its use as food additive and in pharmaceutical applications. Enhancement of capsaicin and related metabolites of the capsaicinoid biosynthetic pathway in Capsicum fruits was achieved using laminarin-an algal cell wall polysaccharide as elicitor. A 1.5-2 fold increase in the overall capsaicinoid content (capsaicin & dihydrocapsaicin) was observed in the fruits of the plants sprayed with aqueous extract of laminarin over the control. Of the concentrations tried 2% (W/V) laminarin spray was effective in enhancing the capsaicinoid and related metabolites. Lower concentration of laminarin, had no significant impact on metabolite profiles. Laminarin could be effectively used as an alternate source for conventionally used several biotic and abiotic elicitors for improvement of secondary metabolites in plants of high economic importance.

Keywords: Biotic elicitor, laminarin, phenylpropanoids, capsaicinoids

---

INTRODUCTION

Capsicum Sps include hot and sweet pepper crops of high global economic value. Oleoresins from fruits of *Capsicum* are useful as pungent food additives and also the food grade pigments are of commercial importance to the food industry [1,2,3]. Capsaicinoids are also well known for their medicinal and pharmaceutical potentialities [4,5,6]. Capsaicinoids are responsible for the pungency of the fruits of *Capsicum* sp. They are biosynthesized by enzymatic condensation of vanillylamine, a phenylpropanoid derivative with several fatty acyl moieties of the leucine/valine based fatty acid pathway [7,8]. The pungency in *Capsicum* varies to a great extent from species/varieties and is regulated by several factors including geographic, environmental and genetic causes [9]. Based on these variations *Capsicum* varieties are popularized regionally each having its unique aromatic, culinary, variable color and pungency related properties and thus are a highly consumed vegetable/spice commodity worldwide [3,10,11]. Unlike capsaicinoids, a class of nonpungent analogues *viz* capsinoids are found in several sweet pepper varieties and are gaining medicinal importance for fat/lipid metabolism capabilities in recent days [12,13,14]. We have previously reported the use of biotic and abiotic elicitors showing its influence on capsaicinoid and related secondary metabolites [15,16]. In one such approach we have used the seaweed based polysaccharide (laminarin) as a biotic elicitor to study its efficacy in enhancement of capsaicinoids.

Seaweeds and the byproducts related to it have found its wide application in agricultural practices [17] and in plant protection practices offering resistance against pathogenic infections [18]. Laminarin a brown algae derived storage polysaccharide, chemically a linear β-1,3 glucan (25-50 glucose molecules linked by β-1,3 glucoside bond) is well known for its induction of defense response and resistance against several pathogens, on its application in plants

[19]. It has been shown that these oligosaccharides in its depolymerized form upon application triggers methyl jasmonate (MeJa) and salicylic acid (SA) pathways, PAL (Phenylalanine ammonia lyase), LOX (Lipoxygenase) enzymes which in turn stimulate several defense related genes involved in plant protection [19,20]. Laminarin was found to reduce infection of soft rot pathogen *Erwinia carotovora* in tobacco leaves [21]. Laminarin in its sulfated (PS3) form is found to induce H_2O_2 production at sites of downy mildew infected grape plants further triggering upregulation of several defence related genes, phenols and callose [22]. In a study carried out by Allègre *et al*., [23] on *Plasmopara viticola* infected grapevine leaves it was found that PS3 conferred 76% of resistance towards the infection avoiding sporulation of the fungus. There are no reports available on influence of laminarin on secondary metabolites production in plants except a recent report wherein, an increase the annatto pigment profile in *Bixa orellana* was achieved by using laminarin as an elicitor [24]. In the present study, we have found the application of laminarin as a biotic elicitor for enhancing secondary metabolite responsible for pungency in *Capsicum frutescens.*

MATERIALS AND METHODS

Plants of *Capsicum frutescens* (bird's eye chilli) were established in pots in poly house at Plant Cell Biotechnology Department of the Institute. All the standards used in this study *viz*., laminarin, vanillylamine, ferulic acid, capsaicin and dihydrocapsaicin were procured from Sigma, USA. Aqueous extract of laminarin (10%w/v) was used as a stock before spraying at different concentrations to the flowers. Three different concentrations 0.5%, 1%, 2% diluted with distilled water were sprayed to the flowers during anthesis. Plain distilled water was used as a control. Elicitor sprayed flowers were cautiously tagged with labels. Fruits were harvested at intervals of 10 days up to 50th day after anthesis. Hence forth referred to as Days after flowering (DAF).

Analysis of metabolites

Analysis and quantification of phenylpropanoid intermediates and capsaicinoids were carried out as per the method of Prasad *et al*., [16]. In brief the harvested fruits were dried to 60ºC until it attained constant weight. The dried fruits were homogenized with acetonitrile in a mortar. The extract was centrifuged at 10,000 rpm at 4ºC for 10min and pallet was discarded. The supernatant was dried *in vacuo* and resuspended in methanol before analysis using HPLC as reported by Prasad *et al*., [16].

Statistical analysis

The experiment was performed in a randomized block design with five replicates. For each treatment fifty flowers were sprayed. Five fruits tagged for each treatment were taken for capsaicinoid and related metabolite analysis at 10 DAF intervals up to 50 DAF. Data obtained were subjected to statistical analyses for the significance of the study using one-way analysis of variance using Microsoft Excel XP ® (Microsoft Corporation, Washington).

RESULTS AND DISCUSSION

Growth and fruiting pattern of *C. frutescens* was prior studied for determining the elicitor treatment schedule. Aqueous solution of laminarin in different concentrations was sprayed to the floral buds during anthesis. Metabolites of the capsaicinoid biosynthesis pathway were analysed until complete maturation of the fruits (50DAF). Analysis of the elicitor treated fruits showed a progressive increase in the levels of phenylpropanoid metabolites *viz* ferulic acid and vanillylamine along with the final products of the capsaicinoid biosynthesis pathway-capsaicinoids which was evident under laminarin treatment in *C. frutescens* **(Figure 1a)**. Laminarin application showed enhancement in the level of ferulic acid compared to control fruits.

Maximum level of ferulic acid was recorded at 50th day (48.78 ± 1.34µmol/g DW) at highest concentration of laminarin spray (2%) which was 4 fold over the control **(Figure 1a)**. It could be noted that at any stage from the 30th DAF there was four fold higher levels of ferulic acid compared to the control in 2% laminarin sprayed fruits. Enhancement of ferulic acid at lower concentration of laminarin was not significant.

Vanillylamine a precursor for all capsaicinoids being phenylpropanoid counterpart of the capsaicinoid pathway also showed nine fold increase (44.57 ± 1.22µmol/g DW) at 20th DAF compared to control in 2% laminarin treated fruits **(Figure 1b)**. Overall an increase of 1.5- 2.25 folds in vanillylamine content was noted during later stages of fruit growth at the concentrations of elicitor applied.

Figure 01: Effect of Laminarin spray on Ferulic acid profile in fruits of *C.frutescens*
DAF-Day after flowering, Mean ±SE, n=5

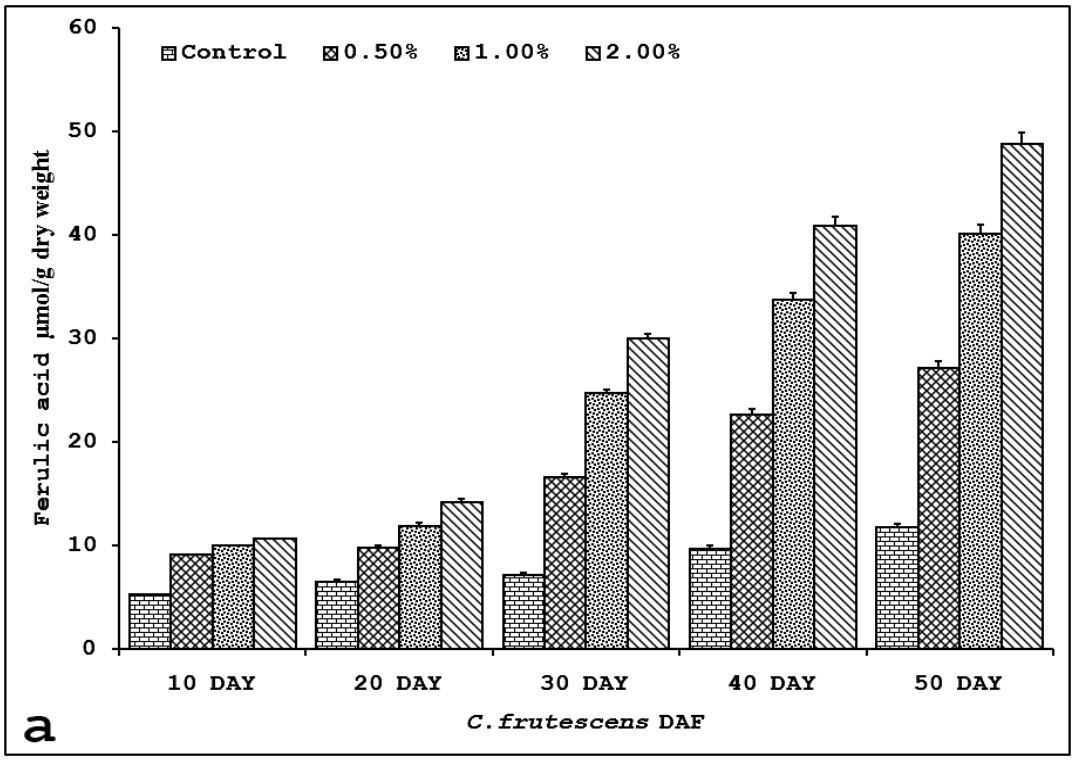
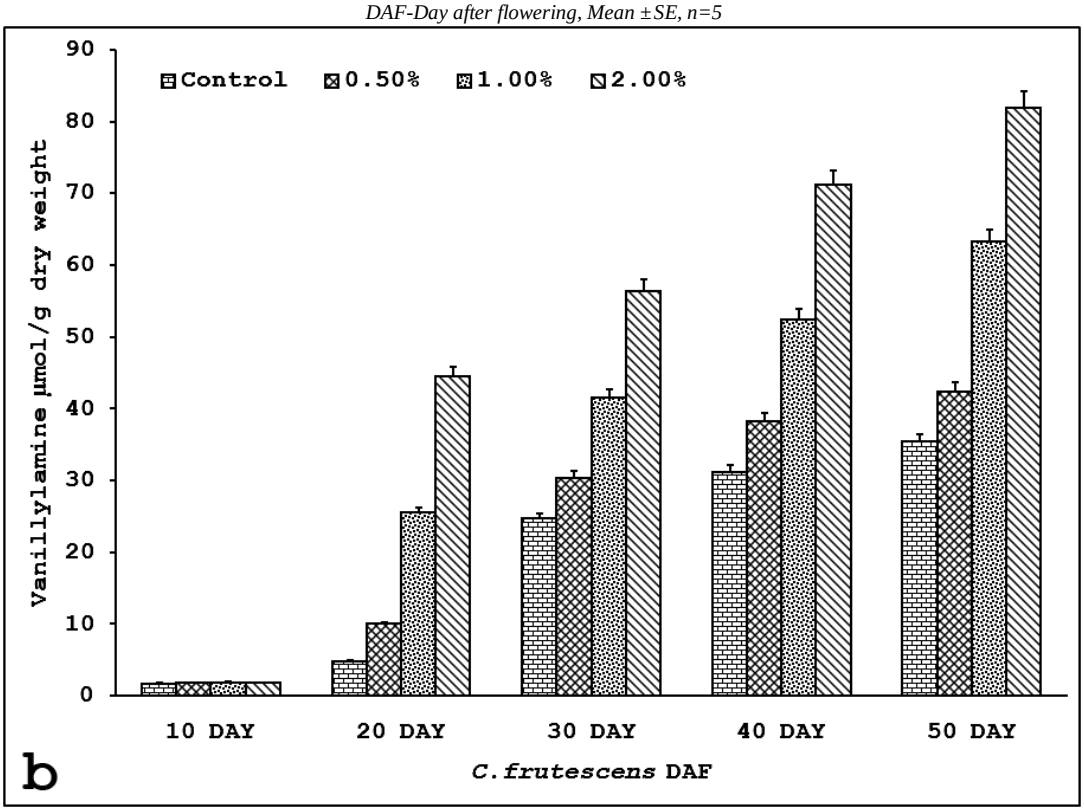
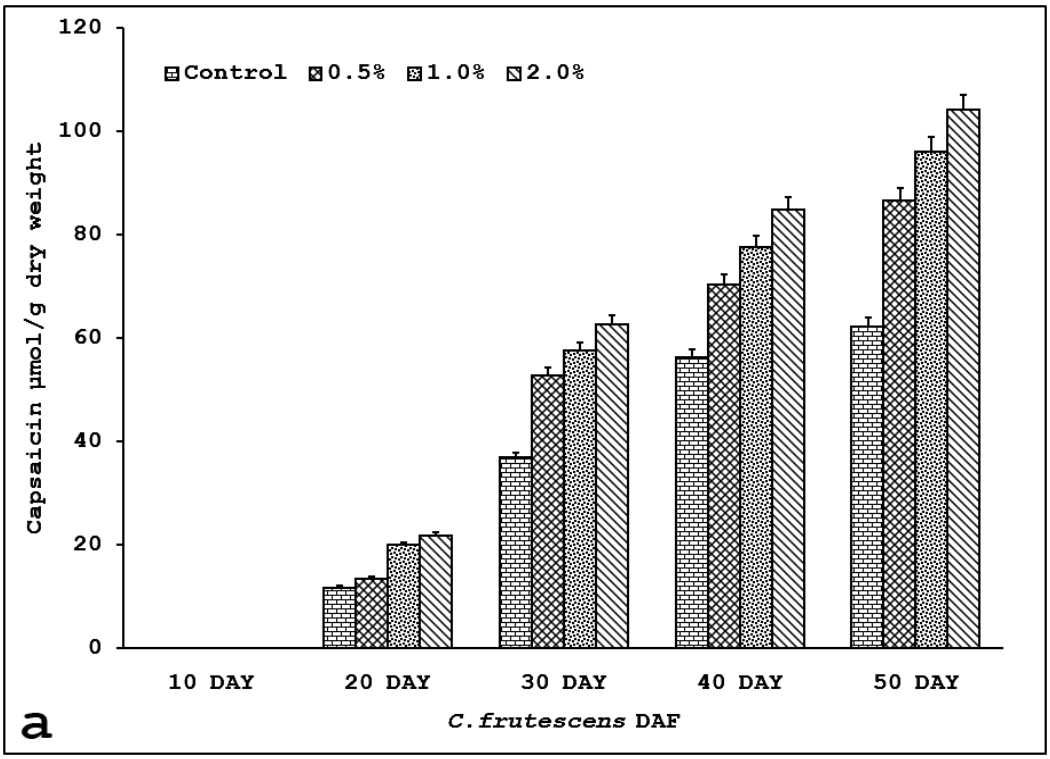


Figure 01: Effect of Laminarin spray on Vanillylamine profile in fruits of *C.frutescens*
DAF-Day after flowering, Mean ±SE, n=5



Capsaicin levels were found to increase by 1.7 folds (62.65 ± 1.72µmol/g DW) at 30th DAF compared to control in 2% laminarin sprayed fruits **(Figure 2a)**. As observed for the previous two metabolites there was a progressive increase in capsaicin accumulation with increasing concentration of elicitor during the ontogeny of fruit. Highest accumulation of capsaicin was at the 50th DAF, in fully mature fruit (104.17 ± 2.86µmol/g DW). However dihydrocapsaicin accumulation was at its peak on 20th DAF recording 2.6 fold enhancement over the control. Overall an increase in DHC concentration up to 1.6 fold was achieved at highest level of elicitor treatment **(Figure 2b)**.

Figure 02: Effect of Laminarin spray on Capsaicin profile in fruits of *C.frutescens*
DAF-Day after flowering, Mean ±SE, n=5

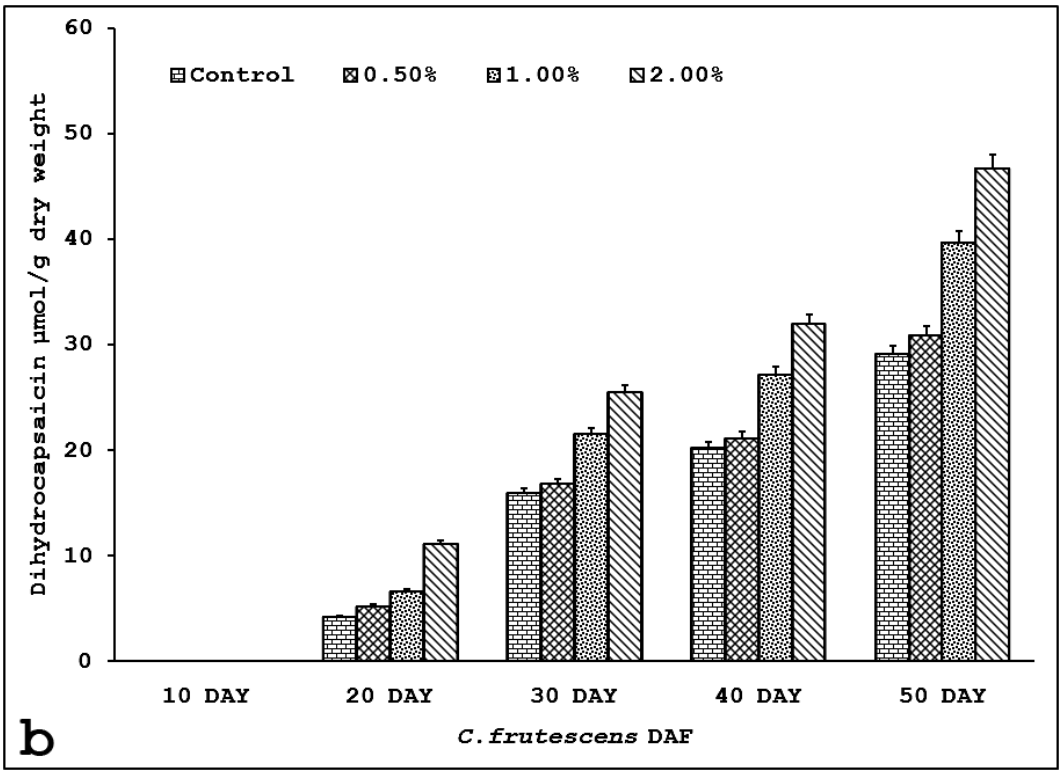


Ferulic acid is the major metabolite of the phenylpropanoid pathway (PPP) in plant systems being an entry point for several intermediates and diversions to other pathways. In the capsaicinoid pathway ferulic acid is the precursor of vanillin which further influenced by a transaminase reaction converts into vanillylamine. In the present study, there was a significant upward increase in ferulic acid content under laminarin treatment, which would further lead to an increase in downstream metabolites pools. This was evident with reference to both vanillylamine and also capsaicinoids especially at matured stage of fruit (20-50 DAF). The foldwise decrease in vanillylamine level at later stages of fruit development in contrast to early stages is plausibly due to its rapid utilization for capsaicinoid biosynthesis. As found in potato and tobacco it could be hypothized that laminarin based elicitation induces over expression of phenylpropanoid pathway (PPP) thereby a cascade of events lead towards enhancement of capsaicinoids [21, 25].

The enhancement of PPP intermediates and capsaicin content in *C. frutescens* in our study was supported by earlier report, which revealed the efficiency of laminarin as an elicitor to induce defense response [26,27], combating fugal infestation and also to improve the crop yield. It is known that fungal cell wall based elicitors improve the secondary metabolites production [27]. It is reported that application of β-1,3 glucooligosaccharide (laminarin) as elicitors causes increase in metabolic flux in phenylpropanoid pathway in potato tuber tissues [25]. We have earlier shown the increase in PPP metabolites by application of both abiotic and biotic elicitors. Spraying of mycelia extracts of fungal isolates (*Rhizopus* and *Aspergillus*) had a profound impact on enhancement of capsaicinoids and related secondary metabolites [15,16]. Similarly usage of abiotic elicitors like MeJa and SA in cell suspension cultures of *C. frutescens* showed increase in levels of capsaicinoids and polyamines [29]. Suresh and Ravishankar, [30] have shown MeJa induced elicitation of PPP metabolites using normal root cultures of *Capsicum frutescens*.

However in the current investigation we have applied elicitor *in planta* for understanding on field performance of such treatments using better alternatives for existing non affordable several abiotic elicitors. Laminarin could be a good alternative source of elicitor as it is economical, environment friendly and also offer support of resistance to plants during infection.

Figure 02: Effect of Laminarin spray on Dihydrocapsaicin profile in fruits of *C.frutescens*
DAF-Day after flowering, Mean ±SE, n=5



CONCLUSION

The result of the current experiment clearly shows the effect and efficacy of sea weed based elicitor having influence on secondary metabolite production. In the current study we have used bird's eye chilli which is rather a non economical variety as an experimental model. However based on these observations we hypothize that laminarin spray would increase the capsaicin and related secondary metabolites in low pungent and commercially important chilli varieties also.

Acknowledgement
The authors are thankful to Department of Biotechnology, New Delhi for financial assistance and CSIR for senior research fellowship for HBG. The institutional support of the Director, CFTRI is greatly acknowledged.

REFERENCES

[1] V. S. Govindarajan, U. J. Salzerb, *Critical Reviews in Food Science and Nutrition*, **1986**, 23, 207-288.
[2] A.K. de, *Capsicum*: The genus *Capsicum*, Medicinal and aromatic plants-industrial profiles -33, Taylor and Francis, London, **2003**.
[3] I. Guzman, P. W. Bosland, M. A. O'Connell , Heat , color and flavor compounds in *Capsicum* fruit, In: The biological activity of phytochemicals, Recent Advances in Phytochemistry 41, D.R. Gang (Ed), (Springer science + Business media, Springer: New York, Dordrecht, Heidelberg, London), **2011** 109-126.
[4] B. C. N. Prasad, R. Shrivastava, G. A. Ravishankar, *Evidence-Based Integrative Medicine*, **2005**, 2, 147-166.
[5] A. A. Oyagbemi, A. B. Saba, O. I. Azeez, *Indian Journal of Cancer*, **2010**, 47, 53-58.

[6] M. d. L. Reyes-Escogido, E. G. Gonzalez-Mondragon, E. Vazquez-Tzompantzi, *Molecules*, **2011**, 16, 1253-1270.
[7] D. J. Bennett, G. W. Kirby, *Journal of Chemical Society C*, **1968**, 442-446.
[8] H. Fujiwake, T. Suzuki, K. Iwai, *Agriculture and Biological Chemistry*, **1982**, 46, 2685-2689.
[9] A. Rodriguez-Burruezo, H. Kollmannsberger, M. C. Gonzalez-Mas, S. Nitz, F. Nuez, *Journal of Agriculture and Food Chemistry*, **2010**, 58, 4388-4400.
[10] J. J. Tewksbury, C. Manchego, D. C. Haak, D. J. Levey, *Journal of Chemical Ecology*, **2006**, 32, 547-564.
[11] Y. Wahyuni, A. R. Ballester, E. Sudarmonowati, R. J. Bino, A. G. Bovy, *Phytochemistry*, **2011**, 72, 1358-1370.
[12] K. Sutoh, K. Kobata, S. Yazawa, T. Watanabe, *Bioscience Biotechnology and Biochemistry*, **2006**, 70, 1513-1516.
[13] F. Kawabata, N. Inoue, S. Yazawa, T. Kawada, K. Inoue, T. Fushiki, *Bioscience Biotechnology and Biochemistry*, **2006**, 70, 2824-2835.
[14] T. Watanabe, K. Ohnuki, K. Kobata, *Expert Opinion Drug Metabolism and Toxicology*, **2011**, 7, 533-542.
[15] B. C. N. Prasad, H. B. Gururaj, V. Kumar, P. Giridhar, R. Parimalan, A. Sharma, G. A. Ravishankar, *Journal of Agriculture and Food Chemistry*, **2006a**, 54, 1854-1859.
[16] B. C. N. Prasad, H. B. Gururaj, V. Kumar, P. Giridhar, G. A. Ravishankar, *Journal of Agriculture and Food Chemistry*, **2006b**, 54, 6660-6666.
[17] D. J. McHugh, A guide to the seaweed industry, *FAO Fisheries Technical Paper 441*, FAO, Rome, **2003.**
[18] J. S. Craigie, *Journal of Applied Phycology*, **2011**, 23, 371-393.
[19] A. Aziz, B. Poinssot, X. Daire, M. Adrian, A. Bézier, B. Lambert, J. M. Joubert, A. Pugin, *Molecular Plant-Microbe Interactions*, **2003**, 16, 1118-1128.
[20] J. Vera, J. Castro, A. Gonzalez, A. Moenne, *Marine Drugs*, **2011**, 9, 2514-2525.
[21] O. Klarzynski, B. Plesse, J. M. Joubert, J. C. Yvin, M. Kopp, B. Kloareg, B. Fritig, *Plant Physiology*, **2000**, 124, 1027-1037.
[22] S. Trouvelot, A. L. Varnier, M. Allègre, L. Mercier, F. Baillieul, C. Arnould, V. Gianinazzi-Pearson, O. Klarzynski, J. M. Joubert, A. Pugin, X. Daire, *Molecular Plant-Microbe Interactions*, **2008**, 21, 232-243.
[23] M. Allègre, M. C. Héloir, S. Trouvelot, X. Daire, A. Pugin, D. Wendehenne, M. Adrian, *Molecular Plant-Microbe Interactions*, **2009**, 22, 977-986.
[24] G. Mahendranath, A. Venugopalan, P. Giridhar, G. A. Ravishankar, *International Journal of Agricultural Environment and Biotechnology*, **2011**, 4, 163-166.
[25] F. Matsuda, K. Morino, R. Ano, M. Kuzawa, K. Wakasa, H. Miyagawa, *Plant and Cell Physiology*, **2005**, 46, 454-466.
[26] N. T. Keen, M. Yoshikawa, M. C. Wang, *Plant Physiology*, **1983**, 71, 466-471.
[27] L. Mercier, C. Lafitte, G. Borderies, X. Briand, Marie-Thérèse, Esquerré-Tugayé, J. Fournier, *New Phytologist*, **2001**, 149, 43-51.
[28] J. Zhao, Q. Hu, Y. Q. Guo, W. H. Zhu, *Plant Science*, **2001**, 161, 423-431.
[29] G. Sudha, G. A. Ravishankar, *Current Science*, **2003**, 85, 1212-1217.
[30] B. Suresh, G. A. Ravishankar, *Plant physiology and Biochemistry*, **2005**, 43, 125-131.