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SHORT COMMUNICATION

EFFECT OF ELICITOR SPRAY AND *ERYSIPHE POLYGONI*, INOCULATION ON β-1, 3 GLUCANASE ACTIVITY IN PEA CULTIVARS RESISTANT AND SUSCEPTIBLE TO POWDERY MILDEW

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Activity of β -1, 3 glucanase in pea cultivars Arkel and JP-179, susceptible and resistant to powdery mildew, respectively was estimated after spray with elicitors [salicylic acid (SA) and 4-aminobutyric acid (4-ABA)] and inoculation with *E. polgoni*. There was a significant increase in enzyme activity as compared to control in both the cultivars after treatment with 5 mM SA, but 5 mM 4-ABA treatment did not show significant effect. Maximum enhancement in enzyme activity was observed after inoculation with *E. polygoni* in both the cultvars. The values of enzyme activity were higher in JP-179 as compared to cultivar Arkel. The protein content increased in the intercellular fluid after above treatments. Since, glucanases are thought to play a direct role in plant defence by digesting fungal cell walls, as suggested by their anti-fungal properties, hence treatment with 5 mM SA may contribute to the acquired resistance of the pea plant by induction of glucanase activity.

Key words **: Acquired resistance, 4-aminobutyric acid, elicitor, glucanase, salicylic acid.**

Plants have developed remarkable strategies to adapt to environmental changes by using a range of constitutive or inducible biochemical and molecular mechanisms (Gachomo *et al.* 2003). Resistance in plants against a disease can be induced as a result of onset of necrosis during plant pathogen interaction (Kuc 1982). In addition to biological induction, elicitation of resistance by certain abiotic substances is of importance for integrating the induced resistance concept in agricultural applications. These elicitors switch on certain favourable biochemical mechanisms in plants (White 1979, Metraux *et al.* 1990). As a consequence of this acquired resistance, plants show enhanced tolerance to a variety of pathogens. One of the important group of enzymes involved in the plants defence against pathogen invasion are hydrolytic enzymes like β-1, 3 glucanase, which have long been suggested as antifugal defence of the plants (De Wit and Spikman 1982, Pierpoint 1986). β-1, 3 glucanase is known to hydrolyse polymers of β-1, 3-linked glucan (Mauch *et al.* 1988). These polymers are major components of the cell walls of higher fungi (Boller 1985). Glucanases are constitutively expressed in most plant tissues at low levels and are induced at both mRNA and protein level upon bacterial, viral, or fungal infection and by elicitors treatment (Wubben *et al.* 1996). The rapid accumulation of β-1, 3 glucanase at the site of penetration in incompatible interaction could play an important role in the defence mechanism of the plant as they hydrolyse β-1, 3 glucans, which are the important structural element of the cell walls of many fungi and thus represent the natural substrate for enzyme. In the hyphal tip of many fungi, β-1, 3 glucans are exposed at the surface (Boller 1987) and could be attacked directly by β-1, 3 glucanases. The oligosaccharides that are released from fungal cell wall as a result of this hydrolytic activity could also function as elicitors of various plants defence responses (Darvill and

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Albershein 1984, De Wit and Roseboon 1980). The spray of exogenous elicitors has been shown to induce the natural defense system of the different groups of the plants against pathogen attack. In the present investigation we have tested the credibility of two abiotic elicitors, viz. salicylic acid and 4-aminobutyric acid in the induction of glucanase in pea plant. The effect of inoculation with the spores of *E. polygoni* on the glucanase enzyme activity and protein content has also been studied in susceptible as well as resistant pea cultivars.

Two garden pea cultivars namely Arkel and JP-179, susceptible and resistant to powdery mildew, respectively were grown in the pots placed in glasshouse under controlled conditions. The plants were watered throughout the experiment. At four weeks old stage the plants were either sprayed with 5 mM elicitor [salicylic acid (SA) and 4-aminobutyic acid (4-ABA)] solution to runoff or inoculated with spores of *E. polygoni* maintained over separate stock of susceptible plants in another room. The fungus *E. polygoni* f. sp. *pisi* was grown on pea cultivar Arkel and conidia were collected. The experimental plants were densely inoculated with conidia of the *E. polygoni* f. sp. *pisi* by dusting. The control plants were sprayed with distilled water only. The intercellular fluid was collected from top four leaves, sampled 24, 48, 72, 96 and 120 h after the treatment following De Wit and Spikman (1982) and was used for glucanase enzyme assay by the method of Abeles and Forrence (1970). The protein content in intercellular fluid was measured by the method of Bradford (1976).

Effect of spraying SA and 4-ABA or inoculation with the spores of powdery mildew on glucanase activity in Arkel and JP-179 cultivars of pea is shown in Table 1. On spraying the leaves of Arkel with 5mM, SA the glucanase activity increased significantly as compared to control plants even after 24 h of treatment. There was a gradual increase in enzyme activity after 24 h to 120 h of treatment. When the plants were sprayed with 5 mM of 4-ABA, there was a slight increase in enzyme activity over the control, however, the values were not significant statistically. When plants were inoculated with the spores of *E. polygoni*, significant and gradual increment in the enzyme activity was observed after different time intervals (24, 48, 72, 96 and 120 h). On comparing the glucanase activity of three different treatments, maximum enhancement in enzyme activity was observed after inoculation of plants with *E. polygoni* spores. Moreover, SA appeared to be a better inducer of enzyme as compared to 4-ABA. Protein content in the intercellular fluid of the leaves also increased after treatment with elicitors or inoculation with *E. polygoni* (Fig. 1A).

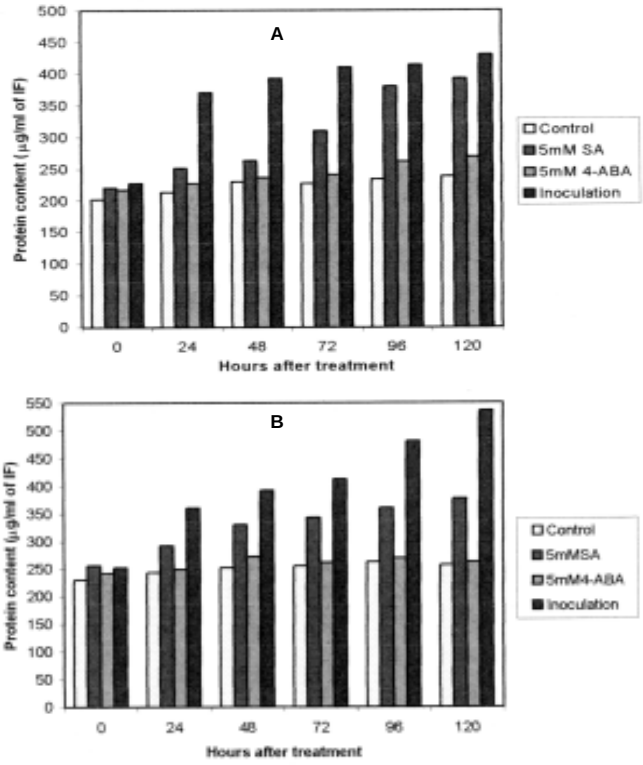

Fig. 1. Protein content in the intercellular fluid (IF) of elicitor (SA and 4-ABA) treated and *E. ploygoni* inoculated leaves of Arkel (A) and JP-179 (B) pea cultivars

It has been reported that 20 or more proteins are newly formed or strongly induced upon infection or elicitor treatment (Cramer *et al.* 1985). The increase in the protein content might be due to induction of synthesis of different enzymes after the treatment. White *et al.* (1987) demonstrated that SA and many of its derivatives are potent inducers of glucanase in broad range of both dicotyledonous and monocotyledonous plants. Dann (1996) also reported increase in β-1, 3 glucanase activity in the extracts of *Phaseolus vulgaris* leaves following treatment with 2, 6-dichloroisonicotinic acid or *Colletotrichum lindemuthianum*. In comparison to Arkel, the control plants of JP-179 cultivar showed higher enzyme activity, suggesting that the higher glucanase activity could be the contributing factor toward resistance against the disease in

Table 1. Effect of elicitors (SA and 4-ABA) spray and *E. polygoni* inoculation on β-1, 3 glucanse activity (mg glucose equivalents formed/h/g fresh wt.) in pea leaves.

Hours after treatment	Control	SA (5mM)	4-ABA (5mM)	Inoculation with fungus *(E. polygoni)*
		Arkel (susceptible)		
0	0.795	0.798 NS	0.797 NS	0.810 NS
	±0.13	±0.16	±0.20	±0.17
24	0.797	1.49**	0.830 NS	2.51**
	±0.09	±0.13	±0.01	±0.02
48	0.852	1.92**	0.890 NS	2.70**
	±0.15	±0.04	±0.07	±0.09
72	0.844	2.43**	0.893 NS	2.81**
	±0.02	±0.05	±0.09	±0.15
96	0.845	2.72**	0.913 NS	2.93**
	±0.08	±0.07	±0.01	±0.13
120	0.840	2.75**	0.976 NS	2.98**
	±0.03	±0.04	±0.07	±0.12
		JP-179 (resistant)		
0	1.30	1.31 NS	1.36 NS	1.37 NS
	±0.12	±0.12	±0.09	±0.09
24	1.28	1.72*	1.30 NS	2.73**
	±0.02	±0.19	±0.04	±0.04
48	1.36	1.96**	1.39 NS	3.54**
	±0.12	±0.12	±0.03	±0.04
72	1.26	2.30**	1.29 NS	3.60**
	±0.03	±0.04	±0.05	±0.05
96	1.20	2.80**	1.26 NS	3.63**
	±0.04	±0.09	±0.04	±0.04
120	1.28	2.92**	1.27 NS	3.67**
	±0.07	±0.31	±0.07	±0.13

*and **, $P < 0.05$ and $P < 0.01$ respectively
Values are the mean of three replicates ± S.D.

this cultivar. Inoculation of the plants with the *E. polygoni* resulted in increase in enzyme activity over the control and was significant after 24 h. The protein content in the intercellular fluid also increased after these treatments (Fig. 1B).

The study thus indicated that the 5mM SA and inoculation with *E. polygoni* increases the glucanase activity in both the cultivars but JP-179 cultivars has higher values of enzyme activity than the Arkel, which could be one of the factors responsible for defence. Abeles *et al.* (1971) also reported that ethylene treatment induces β-1, 3 glucanase and chitinase activities in bean leavs. As many fungi have cell wall rich in chitin and β-1, 3 glucans, they hypothesized that these two hydrolases might help the plants to defend against pathogenic fungi.

It was shown in a number of plant tissues that chitinase and β-1, 3 glucanase are induced not only by ethylene but also by pathogen attack and by elicitors (Mauch *et al.* 1984, Roby *et al.* 1986). The values for glucanase activity after spray with 5 mM SA in Arkel was close to the one after the fungal inoculation, indicating that SA spray has the tendency to mimic the action of fungal inoculation, which provokes the defence system of the plants. The glucanase activity in 5 mM SA sprayed Arkel was close to the control plants of JP-179 which is resistant to fungal infection. The study, thus, suggested that the spray of susceptible pea cultivars with 5 mM SA could increase the glucanase activity, providing more capacity to the plant for hydrolysing the β-1, 3 glucan component of fungal cell wall thus, contributing towards resistance.

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