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Full Length Research Paper

Induced systemic resistance and promotion of wheat and barley plants growth by biotic and non-biotic agents against barley yellow dwarf virus

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Barley yellow dwarf virus (BYDV) is an important virus infecting wheat and barley plants and transmitted by several species of aphids in Iraq. *Pseudomonas fluorescence* and *Azospirillum irakense* at 10^8 CFU/ml, Sea force extract and Elsa fungicide at 1 ml/L were used to induce resistance in the plant against BYDV. The four elements were applied before and after virus-plant inoculation. Results show that all elements stimulated plant growth as estimated by plant heights and chlorophyll concentrations, and elicited significant reduction in disease incidence as determine by BYDV-disease scoring symptoms. The applications of these elements twice (before and after virus inoculation) were found to be more efficient in promoting plant growth and reducing virus disease score. The plant heights, chlorophyll concentrations and BYDV-disease scores were 82.25, 85.59, 74.38, 76.26 cm, 54.19, 45.81, 47.98, 47.85 µg/cm^2 and 2.0, 3.2, 2.8, 3.3 for *P. fluorescence*, *A. irakense*, Sea force extract, and Elsa fungicide treatments, respectively as compared to 62.08 cm, 38.10 µg/cm^2 and 1.2 in control treatments for the same parameters. *P. fluorescence* was more efficient in reducing disease incidence (2.0) as compared to 3.2 with *A. irakense*, 2.8 with Sea force extract, 3.3 with Elsa, and 5.4 with control. The partially resistant lines, (IBA 99, Arivate and Karonea) were found to be more responsive to treatments than the susceptible ones (Hashmia and Kara).

Key words: Sea force extract, barley yellow dwarf virus, *Azospirillum irakense,* Elsa fungicide.

INTRODUCTION

Barley yellow dwarf virus (BYDV), Luteovirus, (Luteoviridae), is an important virus infecting wheat and barley cultivation and transmitted by several species of aphids in Iraq. The virus was characterized by biological and serological means in previous study (Al-Ani et al., unplished). Due to inefficacity of chemicals to manage the virus disease, the research was oriented towards searching for compounds capable of inducing systemic resistance in the plant against the virus.

It has been reported that the plant posses numerous defense mechanisms to protect themselves against pathogens attack. Some of these mechanisms are constitutive include preexisting physical and chemical barriers of the cell wall (Boweles, 1990; Ton et al., 2002).

Other mechanisms are inducible and become activated after pathogen infection including synthesis of small molecules (phytoalexins), deposition of callose around the penetration site, cell collapse around the site of infection (hyper sensitive reaction) and production of anti-pathogen proteins (Mehdy, 1994; Jackson and Taylor, 1996). One of the inducible mechanisms is activated after infection by necrotizing pathogen inducing cell collapse around site infection, rendering other uninfected plant parts resistant to several pathogens (Pieterse et al., 2001). This resistance is referred to as systemic acquired resistance (SAR) (Ryals et al., 1996). SAR is characterized by increase of salicylic acid (SA) synthesized and the activation of pathogenesis-related proteins (PRP) (Malamy et al., 1990; Kloepper et al., 1992; Van Loon et al., 1998).

Another type of resistance induced by non-infectious microorganisms is present on plant root surface. Certain microorganisms were isolated from naturally disease-suppressive soil, mainly *Pseudomonas* spp. that

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promoted plant growth by suppressing soil borne pathogens and inducing systemic resistance in the above ground plant parts (ISR) (Vanpeer et al., 1991; Van Loon et al., 1998). It was shown that ISR is independent of SA (Pieterse et al., 1996; Press et al., 1997; Van Loon et al., 1998), but is dependent on Jasmonic acid (JA) and ethylene (Pieterse and Van Loon, 2007). It was reported that ISR may be induced also by various natural products such as plant extracts to subsequent pathogen attack (Walters et al., 2005).

Several studies have been shown that *Pseudomonas* spp. can trigger plant-mediated resistance in the above ground plant parts (Van Loon et al., 1998; Pieterse et al., 2001; Walters, 2010).

The objective of this study was to evaluate the efficiency of *Pseudomonas fluorescence* and *Azospirillum irakense*, the organic product Sea force and the fungicide Elsa in reducing barley yellow dwarf virus disease incidence in treated plants.

MATERIALS AND METHODS

The virus, BYDV isolate, identified in previous study at Plant Protection Department, College of Agriculture, University of Baghdad, was used in this study. The virus was transmitted from BYDV-infected barley plants to healthy ones by *Macrosiphum avenae*. Apterus of *M. avenae* were transferred from corn plants onto healthy barley plants in cages (36 × 27 × 53 cm) in a glasshouse for rearing. Groups of these aphids were transferred onto virus infected barley plants and maintained in insect proof glasshouse (12 to 25°C) for 24 h. The aphids were then transferred onto healthy wheat and barley plants (10 aphids/plant) for 48 h. The inoculated plants were maintained in the glasshouse until symptoms development. The plants exhibiting symptoms were used as virus source.

Three breeding lines of wheat, IBA 99, Karonea, Hashmia, and two of barley, Arivate, Kara, (IBA 99, Arivate and Karonea were resistant to BYDV, Hashmia and Kara were susceptible) provided by IBA research center were used in this experiment. Seeds of these lines were surface sterilized with 2% sodium hypochlorite for 30 s, then rinsed in sterilized distilled water and air dried. The seeds were sown in autoclaved soil/peat mixture (3:1) in pots of 20 × 30 cm under glasshouse conditions, 12-25˚C.

The bacteria, *P. fluorescence* was isolated from potato rhizosphere soil and grown on King's medium B agar plates, previously autoclaved at 121°C and 1.5 kg/cm^2 for 15 min. The plates were incubated at 23°C for 48 h, and isolated colonies were cultured in liquid King's medium B in Erlenmeyer flasks of 250 ml, one colony/flask. The flasks were agitated for 10 min and incubated at 23°C for 48 h. The number of colony forming unit (CFU/ml) was calculated by plate count method on King's medium B agar.

A. irakense was obtained from Plant Pathology Department, College of Science, University of Baghdad, and was previously isolated from maize rhizosphere soil. The bacteria were grown on nutrient agar plates at 35°C for 24 h. Isolated colonies were cultured in nutrient broth in Erlenmeyer flasks autoclaved at 121°C and 1.5 kg/cm^2 for 20 min (2 colonies/flask). The flasks were incubated at 35°C for 48 h and the CFU/ml was calculated as earlier mentioned.

Seaforce extract was obtained from Debbane Co. Lebanon, produced by Dupont Co. and used at 1 ml/L water.

The fungicide Elsa, carbendizim (Methylbenz imidazole-2-YL Carbamate), belong to benzimidazole group, and is a systemic fungicide that acts on nucleic acid.

Treatments

The bacteria were applied at three stages, before virus inoculation by watering the soil soon after sowing, after inoculation by spraying the plant with bacterial suspension at 10^8 CFU/ml at 4 leaves stage, before and after inoculation by watering the soil after sowing and spraying the plants at 4 leaves stage. Sea force and Elsa were applied before inoculation at 2nd leaf stage and after inoculation at 4th leaf stage.

The plants were inoculated with the virus at 3rd leaf stage by *M. avenae*. Plants inoculated with virus, non-treated were sprayed with distilled water and served as control. The experiments were carried out as a complete randomized design with 10 replicates in a glasshouse. The plants were rated for disease incidence 40 days after planting.

Indication if it induced resistance

The resistance induced in the treated plants was followed by BYDV-visual scoring symptoms according to Schaller and Qualset (1980).

RESULTS

Plant growth promoting activity and reduction of BYDV disease scoring symptoms by *P. fluorescence*, *A. irakense*, Sea force extract and Elsa fungicide were observed in both wheat and barley plants (Table 1). The means of plant heights, chlorophyll concentration and virus-disease scoring symptoms were found to be 83.37, 83.00, 72.81, 74.10, 62.08 cm, 52.97, 42.81, 47.81, 45.87, 38.16 µg/cm^2, 2.8, 4.7, 3.6, 3.9, 5.4, for *P. fluorescence*, *A. irakense*, Sea force extract, Elsa fungicide and control treatments, respectively when these elements were applied before BYDV-plants inoculation. Results show that *P. fluorescence*, Sea force extract and Elsa fungicide significantly reduced BYDV-disease scoring symptoms, while the reduction of BYDV-disease scoring symptoms was non-significant with *A. irakense* as compared to control (4.7 and 5.4), respectively.

Similar results were obtained when the resistance elements were used after virus inoculation but slightly lower than those obtained before virus inoculation (Table 2). It was found that the means of plant height were 77.21, 80.46, 69.65, 71.44 and 62.08 cm, chlorophyll concentration, 51.66, 40.85, 46.82, 44.70 and 38.16 µg/cm^2, virus disease scoring symptoms, 3.3, 5.0, 4.0, 4.18 and 5.4 for *P. fluorescence*, *A. irakense*, Sea force extract, Elsa fungicide and control treatments, respectively. Although, *A. irakense* simulate higher value of plant heights, it failed to reduce virus disease scoring symptoms significantly.

Conspicuous significant increase in plant heights and chlorophyll concentration together with significant decrease in virus disease scoring symptoms were observed when the elements were used before and after virus inoculation: 82.25 cm, 54.19 µg/cm^2, 2.0 with *P. fluorescence*, 85.59 cm, 45.81 µg/cm^2, 3.2 with *A. irakense*, 74.38 cm, 47.98 µg/cm^2, 2.8 with Sea force extract, and 76.26 cm, 47.85 µg/cm^2, 3.3 with Elsa fungi-

Table 1. Effect of *P. fluorescence*, *A. irakense*, Seaforce and Elsa, applied before virus inoculation on plant growth parameters and disease incidence of wheat and barley plants.

Treatment	Variety	Shoot height	Chlorophyll concentration	BYDV scoring
	IBA99	107.99	58.80	1.4
	Karonea	102.94	58.00	1.7
P. fluorescence	Hashmia	41.92	44.60	4.4
	Arivate	108.89	58.96	1.7
	Kara	40.11	44.46	4.7
	Mean	83.37	52.97	2.8
	IBA99	108.82	48.00	2.7
	Karonea	104.00	47.59	3.0
A. irakense	Hashmia	42.90	37.28	7.0
	Arivate	110.00	45.12	3.0
	Kara	49.36	36.06	7.7
	Mean	83.00	42.81	4.7
	IBA99	100.50	52.88	2.4
	Karonea	97.99	51.00	2.4
Sea force	Hashmia	33.96	43.90	5.0
	Arivate	102.08	49.90	2.6
	Kara	27.41	41.33	5.4
	Mean	72.81	47.81	3.6
	IBA99	101.90	50.00	2.4
	Karonea	99.30	48.91	2.7
Elsa	Hashmia	36.51	41.14	5.4
	Arivate	102.99	48.28	3.0
	Kara	29.82	41.00	5.7
	Mean	74.10	45.87	3.9
	IBA99	94.50	41.12	2.7
	Karonea	90.31	39.50	3.7
Control	Hashmia	18.50	37.38	8.4
	Arivate	89.50	40.17	3.4
	Kara	17.59	32.62	8.7
	Mean	62.08	38.16	5.4
L.S.D. (5%)		1.46	0.64	1.00

cide as compared to 62.08 cm, 38.16 µg/cm^2, 5.4 for control treatments, respectively (Table 3).

Unlike the application of *A. irakense* before or after virus inoculation, the use of these bacteria before or after virus inoculation revealed significant decrease in virus disease scoring symptoms (3.2 as compared to 5.4 in control treatment).

DISCUSSION

Results of this study demonstrated that all the agents used stimulate plant growth and enhanced plant resistance against BYDV infection as proved by decrease of virus disease scoring system. The more efficient was *P. fluorescence* followed by Sea force extract and Elsa fungicide. *P. fluorescence* are present on plant root surface where root exudates provide nutrients for its growth, so the bacteria stimulate plant growth by suppressing soil-borne pathogen through competition or antibiosis in addition to secretion of growth regulator (Baker et al., 1991; Tuzan and Kloepper 1995; Leeman et al., 1995; Wei et al., 1996). The effect of Sea force extract and Elsa fungicide in growth promotion may be due to its contents of organic compounds and minerals, which may acts as plant growth regulators.

The enhancement of plant resistance against BYDV may be due to activation of genes in the plants encoding

Table 2. Effect of *P. fluorescence*,*A. irakense*, Sea force and Elsa, applied after virus inoculation on plant growth parameters and disease incidence of wheat and barley plants.

Treatment	Variety	Shoot height	Chlorophyll concentration	BYDV scoring
	IBA99	103.00	57.42	2.0
	Karonea	98.92	57.00	2.4
P. fluorescence	Hashmia	40.12	43.70	4.7
	Arivate	106.00	57.18	2.4
	Kara	38.00	43.00	5.0
	Mean	77.21	51.66	3.3
	IBA99	105.32	46.52	3.0
	Karonea	102.11	45.36	3.4
A. irakense	Hashmia	40.04	34.51	7.4
	Arivate	107.64	43.50	3.4
	Kara	47.20	34.33	7.7
	Mean	80.46	40.85	5.0
	IBA99	98.20	50.50	2.4
	Karonea	94.11	50.04	2.7
Sea force	Hashmia	30.00	42.10	5.4
	Arivate	99.90	47.34	3.4
	Kara	26.02	41.12	6.0
	Mean	69.65	46.32	4.0
	IBA99	99.05	49.11	2.4
	Karonea	96.00	47.14	3.0
Elsa	Hashmia	33.39	40.07	5.7
	Arivate	100.00	47.04	3.4
	Kara	28.72	40.12	6.4
	Mean	71.44	44.70	4.18
	IBA99	94.50	41.12	2.7
	Karonea	90.31	39.50	3.7
Control	Hashmia	18.50	37.38	8.4
	Arivate	89.50	40.17	3.4
	Kara	17.59	32.62	8.7
	Mean	62.08	38.16	5.4
L.S.D. (5%)		0.97	0.36	0.7

for proteins which acts directly as antiviral proteins, or indirectly through induction of other genes which lead finally to a systemic resistance in the plants. These proteins may inhibit the replication of viral nucleic acid and stop the synthesis of viral proteins. The mode of action of Sea force extract and Elsa may follow the same mechanism. It has been reported in previous studies that treatment of plants with plant extract, non-pathogenic agents and chemicals can lead to the induction of resistance in the plants to subsequent pathogen attack (Walter et al., 2005). Rhizobacteria has been widely used to trigger plant mediated resistance in the above ground plant parts in several plant species (Duijff et al., 1998; Leeman et al., 1995; Van Loon et al., 1998; Pieterse et al., 2002).

Results also show that the lines IBA99, Arivate and Karonea, partially resistant to BYDV, were more responsive to treatment than Kara and Hashmia, susceptible lines, this may be due to the influence of genotype, in that the partially resistant lines possess inducible defense mechanism against the pathogen attack which is activated by treatment with some inducers. Hijwegen and Verhaar (1994) showed that greatest protection against powdery mildew on cucumber was obtained in partially resistant cultivar upon treatments with 2, 6-dichloro-isonicotinic acid. Similar finding was also obtained in soybean (Dann et al., 1998).

Further stimulation of plant growth and reduction in

Table 3. Effect of *P. fluorescence*, *A. irakense*, Sea force and Elsa, applied before and after virus inoculation on plant growth parameters and disease incidence of wheat and barley plants.

Treatment	Variety	Shoot height	Chlorophyll concentration	BYDV scoring
	IBA99	109.59	60.00	0.7
	Karonea	104.40	59.11	1.0
P. fluorescence	Hashmia	43.79	46.08	3.4
	Arivate	110.40	60.14	0.7
	Kara	43.08	45.59	4.0
	Mean	82.25	54.19	2.0
	IBA99	112.17	50.33	2.0
	Karonea	106.00	49.98	2.4
A. irakense	Hashmia	44.50	39.90	5.0
	Arivate	113.96	49.73	2.4
	Kara	51.32	39.09	4.0
	Mean	85.59	45.81	3.2
	IBA99	103.00	54.90	1.7
	Karonea	99.09	53.12	2.0
Seaforce	Hashmia	35.99	43.72	3.7
	Arivate	103.96	52.00	2.0
	Kara	29.82	45.14	4.4
	Mean	74.38	47.98	2.8
	IBA99	104.00	53.10	2.0
	Karonea	101.33	49.98	2.4
Elsa	Hashmia	37.97	43.05	4.7
	Arivate	105.07	50.00	2.4
	Kara	32.92	43.09	4.7
	Mean	76.26	47.85	3.3
	IBA99	94.50	41.12	2.7
	Karonea	90.31	39.50	3.7
Control	Hashmia	18.50	37.38	8.4
	Arivate	89.50	40.17	3.4
	Kara	17.59	32.62	8.7
	Mean	62.08	38.16	5.4
L.S.D. (5%)		1.23	0.66	1.2

disease scoring were observed when the inducers were applied twice (before and after virus inoculation). These results indicate that it is necessary to add the inducers more than one time to sustain expression of resistance in the plants against the virus.

Conclusions

BYDV is an important virus in wheat and barley plants in Iraq. Several elicitors, biotic (rhizobacteria) and non-biotic (Sea force and Elsa), can induce systemic resistance in plants and stimulate plant growth parameters. Booster application of the elicitors has been shown to increase the efficacy of plant growth promotion and elicitation of systemic resistance to disease. With the understanding of the mechanism of systemic resistance by different agents, it is possible to use a combination of more than one agent to provide effective protection.

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