Using image analysis to study the allelopathic potential of wheat cultivars against wild barley (Hordeum spontaneum)

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ABSTRACT: Due to increase in the number of herbicide-resistant weeds and environmental concerns in the use of synthetic herbicides, there have been considerable efforts in designing alternative weed management strategies. Crop plants may adversely affect weeds through allelochemicals, which may be released directly or indirectly from live or dead plants. Therefore, the allelopathy potential of nine wheat cultivars against wild barley was evaluated in this study. A special transparent growth box containing black cloth enclosed in it was used to provide a seedbed which allowed the allelochemicals movement along the seed bed and facilitating the use of image analysis. Images were captured every 12 h to evaluate wild barley seedling co-growth with wheat cultivars over time. The allelopathic activities of wheat cultivars affected wild barley growth differently. Falat and Azar2 were more allelopathic cultivars; they inhibited the root elongation of wild barley seedlings by 57.36% and 47.29% respectively. There was clear correlation between root spread of wild barley and seedling growth, suggesting the potential of root spread as an important determinant of allelopathic activity. This method provides a consistent and reliable tool for analyzing the changes in root architecture of allelochemical receiver plants during the exposure to allelopathic neighbors.

Key words: allelopathy; bioassay; image analysis; wheat cultivars; wild barley.

INTRODUCTION

Weed growth is one of the biggest problems in agriculture causing between 10% and 30% of crop yield loss every year (Oerke and Dehne, 1997). Herbicides are often used for weed control, but they can be expensive, can cause environmental problems (Rinaudo et al., 2010) in addition, herbicide resistance, has caused serious problems in weed control (Beckie et al., 2000). As people learn more about possible adverse effects of herbicide exposure, they become more interested in alternative farming systems. Because of these potential problems and increased public pressure on conventional agriculture, there is increasing interest in organic farming systems in around the world (Isik et al., 2009). In organic farming systems no herbicides are allowed (Lammerts van Bueren, 2002). Hence, for the maintenance of crop production, it is essential to develop other methods by which weeds can effectively be controlled (Inderjit and Keating, 1999). Allelopathy is one of the approaches which can be used as an alternative method to combat pests in cropping systems (Inderjit and Keating, 1999). Allelopathy is a natural and environment-friendly technique which may prove to be a unique tool for weed control and thereby increase crop yields (Purvis et al., 1985). Crop varieties with high allelopathic potential are likely to be able to suppress weeds by natural of bioactive allelochemicals into their vicinity, thereby reducing dependence upon synthetic herbicides (Wu et al., 2001).

In 2010, wheat (Triticum aestivum L.) was the second most important cereal crop after maize, and it is a staple food for billions of people of the world (Satorre and Slafer, 1999). Grain yield loss in wheat by weeds is estimated to be 25% (Montazeri et al., 2005). Wild barley (Hordeum spontaneum C. Koch) is a dominant troublesome weed in most wheat fields of Iran (Nevo et al., 1986). Researchers have been demonstrated that wheat allelopathic varietes can be used to reduce weed populations below the threshold level to minimize the applications of herbicides. For example, Bertholdsson (2004) found that certain wheat genotypes can produce toxic root exudates, inhibiting the root growth of perennial ryegrass (Lolium perenne L.) by as much as 50-60%. Wu et al. (2000) showed that wheat accessions differed significantly in their seedling allelopathy, with the
inhibition of root growth of ryegrass ranging from 10% to 91%. Similarly, Rizvi et al. (2004) found that wheat accessions varied in their allelopathic activity in the field and weed growth inhibited by some accessions up to 75%.

Root systems are complex structures that provide functions central to plant fitness, such as water and nutrient acquisition. Plant health and survival are dependent on root system (Lynch, 1995). Curvatures and elongation rates depend on root intrinsic and soil environmental parameters. Because roots can actively direct their growth towards regions of higher nutrient availability and stress-free environment, it is conceivable that root architecture and curvature might be affected in many ways by environmental stresses (Yazdanbakhsh et al., 2010). Plant roots must sense and respond to a variety of environmental stimuli (e.g., Allelochemical) as they pass through the soil. Stimuli may include biotic elements such as fungi or bacteria, and a variety of abiotic factors, including mechanical interactions with obstacles such as rocks and the ever present influence of gravity (Portefield, 2002). Traditional methods of observing root system, such as excavation or washed soil cores, destroy the topology of the root system, but in recent years several newer techniques have been used to nondestructively image root systems (Iyer-Pascuzzi, 2010). The use of image analysis systems has facilitated rapid root length, (spread, area and etc.) measurements (Barnett et al., 1987; Pan and Bolton, 1991; Kirchhof, 1992).

Laboratory bioassay is the first step used to investigate the allelopathy activity of crop variety (Foy, 1999). In such experiments, measuring seedling growth and especially root growth, usually regarded as response. Recent improvements in digital camera technology have made it comparatively easy to acquire large, high-quality image sets detailing the dynamics of root growth (French et al., 2009). The use of image analysis systems, has facilitated rapid root length, spread and surface area measurements (Kirchhof, 1992; Pan and Bolton, 1991). To make this method applicable in allelopathy study high resolution photographs of possible co-growth of donor and receiver plants which are simply taken is desirable. Therefore, we made an effort to evaluate the allelopathic effects of nine more popular wheat cultivars on wild barley and study the root architecture of this weed using image analysis.

MATERIALS AND METHODS

This study was conducted at the University of Zanjan, in 2012.

Preparation of growth box

Two pieces of glass measuring 15 cm by 21 cm with a plastic washer between them attached together using aquarium glue. The plastic washer provided the space for seed bed. A piece of cloth measured 13×18 cm was used as seed bed which put in the space provided between glasses. This allowed the allelochemicals movement along the seed bed. Required moisture was provided with distilled water injected from the bottom of the glass box.

Pregermination of bioassay species

Nine most popular cultivars of Iranian wheat (Azar 2, Falat, Shahriar, Son60, Zagross, Gohar, Maroon, Sardari and Nicknezhad) were studied for allelopathic effect on wild barley in a completely randomized design with four replications. Wheat and wild barley seeds were surface sterilized with 6% sodium hypochlorite for 5 min, rinsed thoroughly with distilled water, and allowed to germinate at 25 °C in incubator for 24 h. Pregerminated seeds of wheat cultivars and wild barley were punched on the seed bed as shown in Fig. 1, then transferred to the glass box. The growth boxes were wrapped with a piece of aluminium foil and placed in a controlled growth cabinet with 25 °C.

Image analysis process

Seedlings photographing started right after the commencement of germination for 10 days. Images of root system were captured per 12 h using a high-resolution (10 Megapixel) digital camera (Canon S95). Data on Root and shoot length over time, angle (the spread of root system) and area of wild barley roots collected using UTHSCSA Image Tool. The spread of wild barley root system determined as suggested by Kujira et al. (1994). After 10 days the wild barley seedlings were harvested shoots and roots were separated, dried for 24 h at 70 °C, and dry weights were measured.

Statistical analyses

The relationship between wild barley root and shoot growth grown adjacent to wheat cultivars with time were described using nonlinear regression. Root and shoot length (y, cm) were fitted to sigmoidal-logistic model:

\[ y = \frac{a}{1 + (\exp(- \frac{(x - x_a)}{b}))} \]

Mathematical expression
where a is the maximum root or shoot length, \( x_0 \) represents the time to 50% of final root length and b is the rate of root or shoot growth.

Nonlinear regression analyses were conducted using SigmaPlot 11.0 (SigmaPlot 2008). Data on wild barley root dry weight, spread and area were subjected to ANOVA using PROC GLM procedure of SAS, version 9.0 (SAS Institute 2002). Means were separated with the SE.

RESULTS

Seedling growth

The logistic function properly described the cumulative growth of roots and shoots against time. The parameter estimates indicated allelopathic capability of wheat cultivars in suppressing wild barley growth (Table 1). Cultivars Falat and Azar2 had the highest prohibitory on wild barley roots and shoots. The final root length of wild barley were 6.05 cm and 7.48 cm and the final shoot length of wild barley were 9.32 cm and 0 cm, respectively, when grown adjacent to Falat and Azar2 (Figs 3, 4). In converse, cultivars Zagross, Maroon and Son 60 had the least inhibitory effect on wild barley root growth. The longest length of wild barley shoots occurred when they grown with the neighboring Nicknezhad, Wild barley shoot length was 18.40 cm, which it was more than control shoot length (Figs 3, 4).

Table 1. Parameter estimates (SE) for the logistic regression describing the relationship between the root and shoot length of wild barley as function of time in response to allelopathic effects of different wheat cultivars.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seedling part</th>
<th>Parameters</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Azar2</td>
<td>root</td>
<td>7.48(0.49)</td>
<td>38.48(7.42)</td>
</tr>
<tr>
<td></td>
<td>shoot</td>
<td>0.00</td>
<td>1.00</td>
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<tr>
<td>Falat</td>
<td>root</td>
<td>6.05(0.56)</td>
<td>23.06(9.59)</td>
</tr>
<tr>
<td></td>
<td>shoot</td>
<td>9.32(1.51)</td>
<td>34.57(11.97)</td>
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<tr>
<td>Gohar</td>
<td>root</td>
<td>12.64(1.61)</td>
<td>28.77(1.38)</td>
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<tr>
<td></td>
<td>shoot</td>
<td>17.55(0.43)</td>
<td>37.23(1.78)</td>
</tr>
<tr>
<td>Maroon</td>
<td>root</td>
<td>13.63(1.50)</td>
<td>34.46(3.57)</td>
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<tr>
<td></td>
<td>shoot</td>
<td>9.91(0.67)</td>
<td>35.26(4.84)</td>
</tr>
<tr>
<td>Nicknezhad</td>
<td>root</td>
<td>12.70(0.32)</td>
<td>29.56(2.65)</td>
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<tr>
<td></td>
<td>shoot</td>
<td>18.40(1.02)</td>
<td>38.18(3.49)</td>
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<td>Sardari</td>
<td>root</td>
<td>12.93(0.87)</td>
<td>32.23(6.49)</td>
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<td></td>
<td>shoot</td>
<td>10.75(1.29)</td>
<td>32.84(9.70)</td>
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<td>Shahriar</td>
<td>root</td>
<td>12.20(0.21)</td>
<td>32.54(2.13)</td>
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<td></td>
<td>shoot</td>
<td>17.59(0.40)</td>
<td>38.48(1.65)</td>
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<tr>
<td>Son60</td>
<td>root</td>
<td>13.65(1.05)</td>
<td>35.84(7.48)</td>
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<td></td>
<td>shoot</td>
<td>11.81(1.62)</td>
<td>34.76(9.26)</td>
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<tr>
<td>Zagross</td>
<td>root</td>
<td>16.79(0.37)</td>
<td>38.23(1.55)</td>
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<tr>
<td></td>
<td>shoot</td>
<td>14.19(0.17)</td>
<td>30.90(1.21)</td>
</tr>
<tr>
<td>check</td>
<td>shoot</td>
<td>15.89(0.29)</td>
<td>26.30(1.72)</td>
</tr>
</tbody>
</table>

Figure 1. Position of pre-germinated seed of donor and receiver plants on the sheets.
Figure 2. Position of pre-germinated seed of donor (left) and receiver plants (right) on the fabric sheets.

Figure 3. The response of wild barley root length over time to allelopathic effects of different wheat cultivars. (Parameter estimates are given in Table 1).
Figure 4. The response of wild barley shoot length over time to allelopathic effects of different wheat cultivars. (Parameter estimates are given in Table 1).
Figure 5. The wild barley seedling growth as affected by allelopathy from (right) more allelopathic cultivar (Azar2) and (left) less allelopathic cultivar (Son60) at the end of the experiment.

Figure 6. Allelopathic effects of different wheat cultivars on the root dry weight of wild barley (vertical bars indicate SE).

Figure 7. Allelopathic effect of wheat cultivars on the shoot dry weight of wild barley (vertical bars indicate SE).

Figure 8. The root area of wild barley as affected by allelopathy from different wheat cultivars (vertical bars indicate SE).
Area and spread of root

Wheat cultivars affect area and spread of wild barley differently when they were present in the growth medium. Wild barley root area in the absence of wheat cultivars (check) was 3.24 cm². Cultivars Azar2 and Falat reduced the average root area of wild barley down 1.34 cm² and 1.44 cm² respectively. However, other cultivars were almost no effect on the root area, Shahriar and Zagross increased the wild barley root area, up to 4.67 cm² and 4.51 cm² respectively, which statistically significant differences were found between the respective cultivars and check in the root area (Fig. 8).

The spread of wild barley root (angle) decreased severely when the inhibitory effects of allelopathy increased. Azar2 had the highest effect on root spread, and wild barley root spread decreased to 32.47°, while, this value was 104.81° in the absence of allelopathy effects (check). Between nine wheat cultivars, Shahriar had the least inhibitory effect on the wild barley root spread (97.74°) (Fig. 9).

DISCUSSION

In this study, the allelopathic activity of nine wheat cultivars on wild barley growth was demonstrated in a laboratory condition. However, as shown, the released substances from wheat cultivars seeds affected wild barley's early growth differently. The wheat varietal differences in the concentrations of allelochemicals in the shoots, roots and root exudates reported by Wu et al. (2002) might account for the variations of varietal allelopathy in wheat. Wu et al. (2001) reported that wheat varieties differ in allelopathic potential against weeds. Kato-Noguchi and Ito (2001) also reported different allelopathic potential among rice cultivars. Weed-suppressing wheat cultivars can be used to naturally suppress weeds (Bhowmika and Inderjit, 2003). In this study, Falat and Azar2 inhibited the elongation of roots of wild barley seedlings by 57.36% and 47.29%. These results indicate that these cultivars with high allelopathic potential can be used to decrease the germinating capacity and lower establishing of wild barley especially in the areas in which the high infestation of wild barley is limiting factor for wheat production.

The root system plays important role in plant adaptation to edaphic limitations and biotic and abiotic stimuli (Yan et al., 1995). The measurement of radical elongation and root dry weight (Wu et al., 2001) are commonly used to determine allelopathic activity; however, interpretation of such data requires some caution, because it may be confounded by delays in germination. Because roots can actively direct their growth towards regions of higher nutrient availability and stress-free environment, it is conceivable that root architecture and curvature might be affected in many ways by nutrient availability, stress environment (Yazdanbakhsh et al., 2010) and allelochemical presence. Our result showed that the spread of receiver plant roots (as indicator for root architecture and curvature) significantly affected by donor plant and there was clear correlation between spread and length of root, suggesting the spread of root as an important determinant of allelopathic activity. This measurement was made using a photo that simply taken at the end of experiment. Simplicity, accuracy and quickness are the major factors of this method to evaluate and analyse allelopathic interactions between neighboring germinating seeds.

Allelochemical slow diffusive movement through the cloth piece between neighboring seedlings makes the conditions more similar to a natural environment. With this method, the inhibitory effect of the donor species on the root and shoot growth of the receiver species can be closely monitored over time. Allelochemicals effect only on neighboring plants placed at a very close distance. Thus, the distance between donor and receiver seedlings is considered in allelopathic capability. The growth box introduced in current study provides manipulation the distance between neighboring plants to estimate the critical distance for receiving the allelochemical. It also provides collecting information on the dynamic of root spread or area, with closely
monitoring the growth. This method has potential to apply in other biological studies such as analysis of root curvatures and elongation rates in roots growth studies.

REFERENCES