International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2756-326X Vol. 7 (1), pp. 001-005, January, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A study of the effect of Cercospora leaf spot at different cultivars and fertilization level

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Accepted 12 December, 2020

Cercospora leaf spot (CLS) caused by *Cercospora beticola* is one of the most destructive foliar disease of sugar beets in all sugar beet-growing areas worldwide. In this study, field trials were carried out to determine the effect of CLS at different cultivars and fertilization level. The result showed that level of resistance against *C. beticola* from 20 variables were differed significantly (P<0.05) and sorted KWS0149>BETA356>Hi0940>KWS6167>KWS8138>KWS4121>Hi0166>DVA02234>BETA807> KWS0142> Ma096> KWS9522> IS0436> BSTO2431> Ma097> BETA464> BETA812> KWS9145> Hi0474> Hi0732. But, Strong and weak of same varieties resistance from three locations apart from 100 km away of this trial series were significantly different. Levels of resistance against *C. beticola* from optimized fertilization were significant different (P<0.05). Low nitrogen reduced sugar beet resistant against *C. beticola.* And level of resistance from 20 variables were differd significantly (P<0.05) and were sorted $N_2P_1K_1$> $N_2P\ _2K_1$> $N_2P_2K_0$ > $N_2P_2K_2$> $N_2P_1K_2$> $N_1P_2K_1$> $N_2P_3K_2$> $N_2P_2K_3$ > $N_2P_0K_2$> $N_1P_2K_2$> $N_3P_2K_2$> $N_1P_1K_2$> $N_0P_0K_0$> $N_0P_2K_2$. So, resistance against *C. beticola* improved after balance fertilizing. It is possible to reduce the pathogen appearance by using varieties resistance and balance fertilizing, which enhanced host resistance to soft rot disease in a way.

Key word: Sugar beet, cercospora leaf spot, varieties, resistance, fertilization level.

INTRODUCTION

Cercospora leaf spot (CLS) caused by *Cercospora beticola* is one of the most destructive foliar disease of sugar beets in all sugar beet-growing areas worldwide (Malandrakis et al., 2006). Control of CLS in Greece and other areas in a warm climate and irrigation is based mainly on frequent fungicide applications (Karaoglanidis and Ioannidis, 2010). However, serious problems have resulted from the extensive appearance of fungicide resistant C. beticola isolates to the intensively used benzimidazoles, organotin fungicides and sterol biosynthesis inhibiting triazoles.

Agricultural scientists are becoming aware of the potential contribution of farmers in developing integrated management of crop diseases in general (Bentley and Thiele, 1999). Much disease management practices such as the applications of fungicides and fumigant; focus on controlling pathogens is often too late to be effective, when disease symptoms are apparent. A more reliable approach is to concentrate on the period before infection occurs and encourage conditions that are unfavorable to the pathogen and favorable to the plant (Wolf and Verreet, 2002; Ghorbani et al., 2008).

Various control strategies, including host-plant resistance, resistant cultivars, integrated control and biological control have been developed. Breeding efforts to generate Cercospora resistance in sugar beet started in the 1920s by Munerati (1920). Historically, resistance was introgressed from the wild sea beet, *Beet vulgaris* L. spp. Maritima (Hecker and Helmerick, 1985). Additional resistant accessions were also found in other subspecies of *B. vulgaris* and in other sections of the genus Beta, namely Corollinae, Nanae and Procumbentes (Asher et

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al., 2001). Resistant against *C. beticola* is a quantitative trait based on the additive effects of at least four to five major resistance genes (Smith and Gaskill, 1970). Therefore, sugar beet lines are selected for resistant against *C. beticola* in the greenhouse using artificial inoculation or in regions where natural infection occurs annually, namely Italy and Greece in southern Europe (Byford, 1996). As the climatic conditions in these countries are different from Germany, resistance of sugar beet varieties is influenced by environmental and cultivation factors (Märländer et al., 2003).

However, the exact number of host genes involved is unknown (Weiland and Koch, 2004). Due to highly variable climatic conditions on a single location, resistant cultivars adapted to the different sugar beet-growing areas worldwide where *C. beticola* occurs regularly are available (Byford, 1996; Mechelke, 2000; Pfleiderer and Schäufele, 2000). Host resistance is not efficient to prevent infection by *C. beticola* entirely but reduces the pathogen's development (Rossi et al., 2000). Therefore, sugar beet lines selected for resistant against *C. beticola* are unreliable in different regions and variable climatic conditions in commercial breeding.

Soil conditions for plant growth can influence the occurrence and severity of plant diseases. Managing and exploiting the suppressive effects of the soil environment as part of an integrated control strategy could make a significant contribution to agricultural sustainability and environmental quality (Quimby et al., 2002*).*

In this study, the impact of different cultivars and fertilization levels on CLS disease severity under natural infection in Heilongjiang, China in 2010. In this study, field trials were carried out to determine the effect of CLS at different cultivars and fertilization level. Secondly, resistant against *C. beticola* in different geographic regions were determined.

MATERIALS AND METHODS

Detection of disease resistant against *C. beticola* for sugar beet varieties

Sugar beet cultivars (KWS0142, KWS0149, KWS9145, KWS8138, KWS6167, KWS9522, KWS4121, BETA807, BETA356, BETA464, BETA812, BSTO2431, Ma096, Ma097, Hi0940, Hi0166, Hi0732, Hi0474, DVA02234, IS0436) differing in the level of resistant against *C. beticola* were used in this study. One location with severe disease occurrence in 2009 was selected to determine cultivars resistant against *C. beticola* under natural infection in Heilongjiang, China in 2010. Three locations apart from 100 km away with severe disease occurrence were selected to determine KWS1049 and KWS4121 cultivars resistant against *C. beticola* of different geographic area.

Field trials were sown between mid and end of April with 70 cm distance between rows. The distances between plants within rows in the natural infection trial was 40 cm, and the trials were manually thinned to a density of 49,500 to 52,500 plants ha^{-1} in seedling trays filled with a standard soil. Weed control were carried out according to local standards.

Response of sugar beet against *C. beticola* at different fertilization level

Field experiments with Sugar beet cultivars KWS0149 were conducted in a location of Heilongjiang province in 2010. Soil nutrients of tested field were obtained under large of 0～15 cm. Organic matter was measured, including the contents of organic matter, available nitrogen, available phosphorus and available potassium etc. for the pre-test. The results showed that the organic matter content is medium rate (20.18～50.20 $g \cdot kg^{-1}$) in tested field soils, ranging from (118.12～204.20 mg $N \cdot kg^{-1}$, 7.18～14.32 mg $P \cdot kg^{-1}$, 44.75～139.57 mg $K \cdot kg^{-1}$). Field trials were sown with 70 cm distance between rows. A 140 cm wide protective belt is left without fertilization by using randomized group (every group mean 5.6 m^2) design with 4 replications.

Traditional fertilization and optimized fertilization were using to analyze effect of sugar beet against *C. beticola* at different fertilization level in this study. Nitrogen (N), phosphors (P) and potassium (K) were replaced respectively by using carbamide (N), diammonium phosphate (P), kalium sulfuricum (K).

Fertilizer application rates of traditional fertilization were designed as the treatments of 600 $kg \cdot ha^{-1}$ (240 kg $N \cdot ha^{-1}$,195 kg $P \cdot ha^{-1}$,165 kg $K \cdot ha^{-1}$), 675 $kg \cdot ha^{-1}$ (270 kg $N \cdot ha^{-1}$, 210 kg $P \cdot ha^{-1}$,195 kg $K \cdot ha^{-1}$), 750 $kg \cdot ha^{-1}$ (300 kg $N \cdot ha^{-1}$,255 kg $P \cdot ha^{-1}$,195 kg $K \cdot ha^{-1}$), 825 $kg \cdot ha^{-1}$ (375 kg $N \cdot ha^{-1}$, 270 kg $P \cdot ha^{-1}$,180 kg $K \cdot ha^{-1}$), and 900 $kg \cdot ha^{-1}$ (420 kg $N \cdot ha^{-1}$, 255 kg $P \cdot ha^{-1}$, 225 kg $K \cdot ha^{-1}$).

Fertilizer application rates of optimized fertilization were $N_0P_0K_0$, $N_0P_2K_2$, $N_1P_2K_2$, $N_2P_0K_2$, $N_2P_1K_2$, $N_2P_2K_2$, $N_2P_3K_2$, $N_2P_2K_0$, $N_2P_2K_1$, $N_2P_2K_3$, $N_3P_2K_2$, $N_1P_1K_2$, $N_1P_2K_1$, and $N_2P_1K_1$ (Detailed data refer to Table 4).

Disease assessment

Disease index severity of all individual sugar beet plants per treatment was assessed according to the modified agronomica disease index severity (Vereijssen et al., 2003; Battilani et al., 1990), which covers a scale from 0 (healthy) to 9 (totally destroyed foliage). Disease index severity in each treatment group was estimated in the middle of August, 2010 using a scale of 0 to 9: 0 = no symptoms on fully leaves; 1= few disease spots of most leaves; 3 = most disease spots of most leaves; 5 =most disease spots of most leaves, dead lateral 1 to 3 leaves; 7 = most disease spots of most leaves, dead lateral 3 to 5 leaves; 9 = most disease spots of most leaves, all leaves and leafstalk dead or whole plant dead.

Statistics

Analysis of variance was carried out with the programme SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Significant differences were indicated with different letters for probabilities ($P<0.05$).

RESULTS

Detection of disease resistant against *C. beticola* for sugar beet varieties

Disease index investigation was carried out in the middle of August. Univariate comparisons showed that level of resistance of 20 cultivars had been differed significantly ($P<0.05$) and were sorted KWS0149> BETA356> Hi0940 >KWS6167> KWS8138>KWS4121> Hi0166> DVA0-2234 > BETA807> KWS0142> Ma096> KWS9522> IS0436>

Table 1. The resistance determination of sugar beet varieties against *C. beticola.*

Cultivars	Disease index	Cultivars	Disease index
KWS0142	17.36 ± 0.54^{abcde}	Ma096	19.01 ± 0.89^{bcdef}
KWS0149	13.08 ± 0.49^{a}	Ma097	20.86 ± 1.01^{cdefg}
KWS9145	23.95 ± 2.85^{fgh}	Hi0940	15.31 ± 0.25^{abc}
KWS8138	16.30 ± 0.86^{abcd}	Hi0166	16.79 ± 0.25^{abcd}
KWS6167	15.81 ± 0.25^{ab}	Hi0732	27.61 ± 1.39^{h}
KWS9522	19.14 ± 0.81^{bcdef}	DVA0-2234	16.79 ± 1.31^{abcd}
BETA807	17.04 ± 1.13^{abcd}	BSTO-2431	19.38 ± 1.60^{bcdef}
BETA356	14.62 ± 4.40^{ab}	IS0436	19.26 ± 1.86^{bcdef}
BETA464	21.73 ± 2.51^{cdef}	Hi0474	25.80 ± 2.50^{gh}
BETA812	22.84 ± 1.39^{efgh}	KWS4121	16.54 ± 1.38^{abcd}

Data are treatment means of pooled data ± standard errors. Values of each column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range tests.

Table 2. The determination of sugar beet resistant against *C. beticola* from different geographic area.

Breeds	Different regions	Disease index
KWS1049	1	13.08 ± 0.49^{a}
	2	27.66 ± 0.25^{c}
	3	17.12 ± 1.72^{b}
KWS4121	1	16.54 ± 1.38^{a}
	2	29.14 ± 1.37^{b}
	3	19.35 ± 3.74^{a}

The distance among the three zones (1,2,3) is 100 km; Data are treatment means of pooled data ± standard errors. Different letters for the same assessment date indicate significant different at $P < 0.05$ according to Duncan's multiple range tests.1, 2, 3 for three locations apart from 100 km away.

BSTO-2431> Ma097> BETA464> BETA812> KWS9145> Hi0474> Hi0732 (Table 1).

Three locations apart from 100 km away with severe disease were selected to analyze relationship between KWS1049, KWS4121 cultivars resistant against *C. beticola* and different geographic area. The result showed that level of same varieties resistance from different geographic area were significant different (Table 2).

Response of sugar beet against *C. beticola* at different fertilization level

Traditional fertilization was designed to analyze cultivars resistant against *C. beticola*. The results showed that level of resistant against *C. beticola* from traditional fertilization were not significant different (Table 3).

Optimized fertilization was designed to analyze cultivars resistant against *C. beticola* from different fertilization level. The results showed that level of resistant against *C. beticola* from optimized fertilization were significant different (Table 4). The results showed low nitrogen reduced sugar beet resistant against *C. beticola* and level of resistance from optimized fertilization were sorted $N_2P_1K_1$> $N_2P_2K_1$> $N_2P_2K_0$ > $N_2P_2K_2$> $N_2P_1K_2$> $N_1P_2K_1$> $N_2P_3K_2$> $N_2P_2K_3$ > $N_2P_0K_2$> $N_1P_2K_2$> $N_3P_2K_2$> $N_1P_1K_2$> $N_0P_0K_0$> $N_0P_2K_2$.

DISCUSSION

In this study, we aimed to estimate effect of different cultivars and fertilization level under natural infection against *C. beticola* in Heilongjiang, China. The 20 cultivars resistant against *C. beticola* were evaluated under natural infection in cultivar trial series. The result showed that KWS series varieties had the character of high resistance to disease in Heilongjiang, such as

Table 3. The determination of resistant against *C. beticola* from traditional fertilization.

Sum ($kg \cdot ha^{-1}$)	Carbamide ($kg \cdot ha^{-1}$)	Diammonium phosphate ($kg \cdot ha^{-1}$)	Potassium sulfate ($kg \cdot ha^{-1}$)	Disease index
600	240	195	165	20.99±1.37[a]
675	270	210	195	22.47±1.73[a]
750	300	255	195	21.97±0.99[a]
825	375	270	180	23.21±1.73[a]
900	420	255	225	21.48±1.96[a]

Data are treatment means of pooled data ± standard errors. Different letters for the same assessment date indicate significant different at P＜ 0.05 according to Duncan's multiple range tests.

Table 4. The determination of resistant against *C. beticola* from optimized fertilization.

NPK content	Disease index	NPK content	Disease index
$N_0P_0K_0$	22.48±2.52[bc]	$N_2P_2K_0$	14.79±1.72[a]
$N_0P_2K_2$	24.68±2.76[c]	$N_2P_2K_1$	13.16±1.08[a]
$N_1P_2K_2$	17.69±2.58[ab]	$N_2P_2K_3$	16.30±1.47[a]
$N_2P_0K_2$	17.08±2.22[ab]	$N_3P_2K_2$	18.43±0.40[ab]
$N_2P_1K_2$	15.55±0.85[a]	$N_1P_1K_2$	18.56±0.74[ab]
$N_2P_2K_2$	15.22±1.24[a]	$N_1P_2K_1$	15.86±1.33[a]
$N_2P_3K_2$	16.18±2.04[a]	$N_2P_1K_1$	13.01±1.30[a]

Data are treatment means of pooled data ± standard errors. Different letters for the same assessment date indicate significant different at P < 0.05 according to Duncan's multiple range tests; Carbamide g/ $5.6m^2$: N_0 mean 0.0, N_1 mean 51.6, N_2 mean 103.2, N_3 mean 154.8; Diammonium phosphate g/ 5.6 m^2: P_0 mean 0.0; P_1 mean 54.8; P_2 mean 109.6; P_3 mean 164.3; Kalium sulfuricum g/ $5.6m^2$: K_0 mean 0.0; K_1 mean 50.4; K_2 mean 100.8; K_3 mean 151.2.

KWS0149, KWS6167, KWS8138, KWS0142 and KWS4121. In addition, others varieties had strong resistance to CLS, such as Hi0166, Hi0940, DVA0-2234, BETA356 and BETA807 to offer basis in preventing CLS and in selecting scientific distribution of resistant variety in Heilongjiang.

In three locations apart from 100 km away, relationship between KWS1049, KWS4121 cultivars resistant against *C. beticola* and different geographic area was analyzed. Same varieties resistance from different geographic areas was significantly different (Table 2). Sugar beet resistant cultivars adapted to the different sugar beet-growing areas worldwide where *C. beticola* regularly occurs were available (Byford, 1996; Mechelke, 2000). But, the suppression of plant defence reactions plays a crucial role in causing plant diseases (Bouarab et al., 2002; Hauck et al., 2003). Schmidt et al. (2004) have shown that inducible plant defences are repressed during the development of CLS. Therefore, host resistance is not efficient to prevent infection by *C. beticola* (Rossi et al., 1999, 2000). So, Field identification of sugar beet resistant from different geographic area cultivars against C. beticola needs to further strengthen and expands the area.

As Walters et al. (2005) pointed out; we need to pay attention to factors that are likely to influence the effectiveness of bio-controls in the field. There are evidences which show both a positive and a negative relationship between available plant nutrients and incidence of certain diseases (Ghorbani et al., 2008). Fertilizer application rates of traditional fertilization and optimized fertilization were designed to analyze cultivars resistant against *C. beticola*. The result showed that strong and weak of resistant against C. beticola from different fertilization level of traditional fertilization were not significantly different (P <0. 05) (Table 3).

However, the results showed significant differences among different fertilization level of optimized fertilization (P <0.05) (Table 4). The results showed low nitrogen could reduce host resistant against *C. beticola.* And balance fertilizing could enhance host resistance to CLS. Fertilizer application rates were designed for enhance sugar beet resistant against *C. beticola* as the treatments of adaptive rate (N:P:K=2:1:1).

Abundant nitrogen encourages succulent growth, a prolonged vegetative period, and delayed maturity of the plant, which increases the period of susceptibility to pathogens. Deficient plants are weaker and slower growing, which are also more susceptible to pathogens (Agrios, 1997). The effect of soil nitrogen level on disease development in different agricultural crops has been shown. For example, Sharma and Kolte (1994) suggested that the plants in pots or field plots which received NK (N 90 kg ha^{-1}) + (K 40 kg ha^{-1}) were more resistant to infection than plants which received N (alone) or P (alone) or NP and PK combinations. Such results provide interesting evidence to support the view that balanced soil fertility could lead to better sugar beet resistant against *C. beticola*.

All in all, a comparative study of resistance determination of sugar beet varieties against C*. beticola* is needed to understand better from different geographic area in order to design comprehensive control on CLS. Accumulation of more knowledge regarding control of CLS should stimulate further conversion of conventional systems of sugar beet production, which incorporate agro-ecological strategies to optimize soil fertilization, sugar beet varieties diversity management and more natural systems of disease regulation without incurring much yield.

ACKNOWLEDGEMENTS

Authors wish to thank to Heilongjing Postdoctoral Science Foundation (LRB09-279), Dr. Start-Up fund research of Northeast Agricultural University (2009RC48) and Ministry of Agriculture Key Laboratory Foundation of Cold Crop Physiology Ecology (Northeast Agricultural University) for financial support.

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