Note

A fresh weight-based method for evaluating soybean resistance to red crown rot

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Soybean red crown rot (RCR) caused by *Calonectria ilicicola* is a serious soil-borne disease affecting soybean production and quality. The current visual necrosis-based method for the measurement of RCR severity is prone to subjectivity as well as time consuming and laborious as it requires digging out and washing the roots to remove adhering soil prior to the visual scoring. Using cultivar Enrei, we show that, upon *C. ilicicola* infection, relative fresh weights (RFW; fresh weights relative to non-inoculated control plants) showed a significant negative correlation with visual RCR severity in apical shoot (trifoliolate and above, $R^2 = 0.96$), shoot (unifoliolate and above, $R^2 = 0.82$) and roots ($R^2 = 0.89$). Furthermore, apical shoot RFW efficiently correlated with varying levels of *C. ilicicola* resistance in two test sets containing 37 soybean cultivars and three wild soybean accessions, exhibiting a significant correlation with visual severity ($R^2 = 0.72$ and 0.79 , $p < 0.01$). Taken together, our results suggest that RFW can serve as an index of soybean RCR severity, providing a simple, rapid, consistent, and cost-effective method for evaluating *C. ilicicola* resistance in soybeans.

Key Words: soybean, red crown rot, disease severity, fresh weight, resistance screening.

Introduction

Soybean red crown rot (RCR) caused by the soil-borne fungal pathogen *Calonectria ilicicola* (Anamorph: *Cylindrocladium parasiticum*) is one of the most serious soil-borne diseases worldwide (Crous *et al.* 1993, Padgett *et al.* 2015). Root infection by *C. ilicicola* can occur soon after soybean planting (Kuruppu *et al.* 2004), but initial symptoms in soybean fields appear during mid to late reproductive growth stages (Akamatsu *et al.* 2020, Roy *et al.* 1989, Yamamoto *et al.* 2017). The typical symptoms include leaf chlorosis/necrosis, root and stem decay with reddish-brown or orange-colored perithecia developing on the basal parts of main stems and roots, and precocious defoliation (Akamatsu *et al.* 2020, Roy *et al.* 1989, Yamamoto *et al.* 2017). In severe cases, most lateral roots are lost from the taproots, resulting in poor plant growth and reduction of seed yield and quality (Akamatsu *et al.* 2020, Roy *et al.* 1989, Yamamoto *et al.* 2017). It has been implicated that a toxic metabolite (PF1070A) of the fungus is associated with the virulence of *C. ilicicola* and disease development (Ochi *et al.* 2011). The estimated maximum yield loss in soybean caused by *C. ilicicola* may be as high as 50% (Berggren and Snow 1989, Gao *et al.* 2012, Padgett *et al.* 2015).

In Japan, more than 80% of soybean crop is grown after paddy (MAFF 2014, Sugimoto *et al.* 2012). The clayed nature of such soybean fields, which is favorable for paddy cultivation, makes them prone to short-term waterlogging due to poor drainage (Bajgain *et al.* 2015). These conditions are favorable for RCR development. RCR has thus been identified as one of the factors limiting soybean production in Japan (MAFF 2014). To date, no practically effective fungicides exist for the control of this disease. Therefore, sustainable agricultural management practices and an efficient ecological strategy to control RCR are urgently required.

Several agricultural practices have been employed to handle RCR in soybean, such as delaying sowing, intercropping with non-host crops, biological control, and conventional tillage to reduce inoculum and spore dissemination by *C. ilicicola* (Gao *et al.* 2012, 2014, Kuruppu *et al.* 2004). However, environmental conditions in the field affect the consistency and effectiveness of these practices. Thus, the use of host genetic resistance is considered the most effective and eco-friendly way of reducing yield losses due to RCR incidences in soybeans. Moreover, resistant cultivars can provide disease protection at no additional cost to the grower. While variation in resistance to *C. ilicicola* has long been reported among soybean cultivars, no complete or high-level resistance to *C. ilicicola* has yet been identified (Jiang *et al.* 2020, Kim *et al.* 1998, Nakajima *et al.* 1994). Recently, we reported identification of three wild-soybean accessions that possess a high-level resistance to *C. ilicicola* (Jiang *et al.* 2020). These wild soybean lines

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should serve as valuable genetic resources for developing *C. ilicicola*-resistant soybean cultivars.

A simple and reliable assessment method is essential for genetic screening and identifying resistance to *C. ilicicola* from a large number of soybean germplasms in order to use them in resistance breeding programs. For quantifying plant disease, visual assessment is probably still the most widely used method (Bani *et al.* 2012, Bock and Nutter Jr. 2012, Pilet-Nayel *et al.* 2002), and the current method for RCR evaluation also relies on visual scoring of necrosis (browning) of the roots. However, visual scoring by naked eyes, especially in a large-scale assessment, can result in bias arising from differences in individual judgments, as well as between experimental repeats (Bock *et al.* 2010). This method is also laborious and time-consuming and costly, because it requires digging out and washing the roots to remove adhering soil prior to the visual scoring.

In view of this, the purpose of this study was to develop a simple and consistent laboratory method for measurement of soybean RCR severity. We present a new method that involves simply measuring apical shoot fresh weights of inoculated and non-inoculated control soybean plants.

Materials and Methods

Cultivars

The soybean cultivar Enrei was used in all the experiments. Additionally, 36 local soybean cultivars (kindly provided by Drs. Youhei Nanjou and Fumio Taguchi-Shiobara at Institute of Crop Science, NARO, Japan), as well as three wild soybean accessions (*Glycine soja* Sieb. and Zucc; JP30157, JP30159, and JP36084, NARO Genebank, <http://www.nias.affrc.go.jp/>) recently identified to be highly resistant to *C. ilicicola* (Jiang *et al.* 2020), were used in two validation experiments: the first validation experiment involved 24 soybean cultivars including Enrei and three wild soybeans (Supplemental Table 1); and the second experiment included 14 cultivars including Enrei and three wild soybeans (Supplemental Table 2).

Plant growth

Both cultivated and wild soybean seeds were pre-conditioned in a moisture-saturated plastic box for 24–48 h at 25°C before sowing, to improve and synchronize seed germination. To increase the water permeability of wild soybean seeds, a small portion of the seed coat on the distal end to the hilum was scraped off with a flat file. Seeds were sown in commercially available, pre-fertilized and granulated soil (Nippi No. 1, Nippon Hiryo, Tokyo, Japan) at a depth of 2–3 mm in 65 mm² plastic pots with a depth of 50 mm (180-ml) and a drainage hole. Five seeds were sown per pot, and the top of the pot was covered with a 2 mm layer of pre-fertilized peaty soil Supermix-A (Sakata Seed Corporation, Yokohama, Japan). All the soils used in this work were autoclaved one day before the seed sowing to eliminate any effect from other soil pathogens. Seeded pots

were watered just enough to fully wet the soil and placed in a container. Water was added to the container as necessary to maintain the soil wetness until the termination of the assay.

Pathogen culture and inoculation

The fungus *C. ilicicola* (isolate UH2-1) was kindly provided by Dr. Sunao Ochi (Research Center for Agricultural Information Technology, NARO, Japan), which was isolated from RCR-diseased soybean roots from Sasayama, Hyogo (Jiang *et al.* 2020). Fungal mycelia were grown on potato-dextrose agar (PDA) plates at 25°C for 1–2 weeks or until the fungal mycelial growth reached the edges of the petri plates.

Pathogen inoculation was performed as described previously (Jiang *et al.* 2020). Briefly, five to eight pieces (~5-mm cubes) of PDA with vigorously growing *C. ilicicola* mycelia were placed in a 500-ml flask containing 200 g of wheat bran-vermiculite medium (wheat bran/vermiculite/water 1:1:3, w/w/v) and incubated at 26°C for 10–14 d, until the medium was fully covered by the fungal mycelia. This culture was used as inoculum, and an inoculum-soil mixture was prepared by mixing the inoculum with Nippi No. 1 soil to a density of 0.2–3.2% as stated in the results and figure legends. The mixture was used to fill plastic pot (180 ml) in which five seeds per pot were sown as described above. Seeds were germinated and the seedlings were grown in a greenhouse at 26°C and 50% relative humidity.

Two validation experiments were done under temperature-controlled growth cabinet at 26°C with 16 h of daytime under 250 to 350 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of light intensity and 50% relative humidity for the remainder of the experimental period. The well-controlled conditions in growth cabinet generally resulted in more stable assay results than in the greenhouse. However, on the other hand, it was found that the disease severity tended to be slightly lower than that obtained under greenhouse conditions. Therefore, in these experiments, the inoculum density was increased to 1.2% v/w to obtain disease levels similar to those in the greenhouse assays.

Measurement of disease severity

Fourteen days after seed sowing (days post inoculation, dpi), the shoot (unifoliate and above) and apical shoot (first trifoliate and above) of soybean seedlings (Fig. 1B) were cut and their fresh weights were measured. Subsequently, the seedlings were taken out from the pots and the roots were washed gently with running tap water to remove adhering soil. The roots were cut off at the base of the stem (hypocotyl) and blotted on a towel paper to remove excess water, and their fresh weights were measured. The relative fresh weight (RFW) was calculated as fresh weight of inoculated plants relative to that of the non-inoculated control plants.

The visual disease severity of roots was scored based on

Fig. 1. Impact of *Calonectria ilicicola* inoculation (1%) on growth of soybean plants (cultivar Enrei) at 14 days post-inoculation (dpi). Representative photograph of the whole plant (A), schematic diagram of different parts of plants for sampling (B), plant height (C), shoot fresh weight (D), and root fresh weight (E) of control (uninoculated) and inoculated plant at 14 days post-inoculation (dpi). Values are means $\pm$ SD, $n = 30$. Different letters indicate significant difference at the 5% level according to Tukey's Honest Significant Difference (HSD) Test ($p < 0.05$). There were three replications and each replicate consisted of 10 plants, $n = 30$.

a six-point rating scale (0 to 5) as described previously (Jiang *et al.* 2020, Nishi *et al.* 1999): 0 = no visible symptom, 1 = small brown necrotic lesions on the primary root, 2 = brown necrotic lesions extending all over the primary root and some lateral roots, 3 = $\geq 50\%$ root-loss owing to rot and severe brown necrosis on subterranean stem, 4 = almost all roots rotted and lost, and 5 = seedlings death. Individual seedlings were considered dead if they had withered and fallen down.

Experimental design and data analysis

All experiments were arranged in a completely randomized design with three replications. For two validation experiments, all the accessions for each replicate were placed in a separate container, and the three replicate containers were rotated within the growth cabinet every two to three days to minimize any location effects and equal distribution of light intensity.

The results were subjected to a one-way analysis of variance using Statistical Tool for Agriculture Research (STAR) Version 2.0.1 software (International Rice Research Institute, Philippines). When the result of test indicates significant then mean values were compared by Tukey's honest

significant difference (HSD) test ($p < 0.05$) using XLSTAT. The regression analysis between visual disease severity and RFW was assessed using linear correlation analysis (SigmaPlot version 11.0).

Results and Discussion

Development of a fresh weight-based method for RCR severity measurement

Compared with the non-inoculated control plants, *C. ilicicola* inoculation at 1% density resulted in a remarkable growth reduction in cultivar Enrei (Fig. 1A), manifesting reductions of up to 50% in plant height (Fig. 1C), 33% in shoot fresh weight (Fig. 1D), and 23% in root fresh weight (Fig. 1E). These results demonstrated that the root rot caused by *C. ilicicola* resulted in biomass reduction. Thus, we investigated how the extent of biomass reduction is correlated to the degree of RCR severity. Plant height was not included in the further investigation as it is generally highly affected by growth conditions other than fungal infection, like light, temperature and soil water conditions.

The Enrei seeds were sown in different doses of *C. ilicicola* inoculum ranging from 0.2 to 3.2% (w/v) to produce varying disease severities (Fig. 2). The results showed a dose dependent visual disease severity (Fig. 2A). The concentrations of 0, 0.2, 0.4, 0.8, 1.6, and 3.2% inoculum resulted in disease severity score of 0, 3.0, 3.3, 3.4, 4.0, and 4.5, respectively (Fig. 2B). On the other hand, the RFW of apical shoot, shoot, and roots were significantly reduced in inoculated plants compared to that in non-inoculated plants. The RFW ranged from 1.0 to 0.81, 0.50, 0.51, 0.39, and 0.29 in apical shoot, 1.0 to 0.84, 0.59, 0.63, 0.51, and 0.39 in shoot, and 1.0 to 0.77, 0.67, 0.70, 0.63, and 0.49 in roots, respectively, with the increasing dose of inoculum. In agreement with the previous studies, the incremental plant growth decreased, and root discoloration increased as inoculum concentration of the pathogen increased (Jackson *et al.* 2005, Jiang *et al.* 2016, Raftoyannis and Dick 2002, Shakiba *et al.* 2012). The RFW reduction was most prominent in apical shoot, followed by shoot and roots (Fig. 2B).

Correlation analysis revealed that the reduction in RFW of all the three tissues is significantly negatively correlated with the increase in visual RCR severity, with the correlation coefficients (R^2) of 0.96 ($p < 0.01$), 0.82 ($p < 0.05$), and 0.89 ($p < 0.05$) for apical shoot, shoot, and roots, respectively (Fig. 3). The finding that RFW is significantly correlated to RCR severity, strongly suggests that the RFW can serve as a new alternative index to current visual disease severity.

Among RFWs examined in this study, the RFW of apical shoot showed the highest reduction in response to RCR incidence, as well as the highest correlation coefficient to RCR severity ($R^2 = 0.96$, $p < 0.01$). These results suggest that the RFW of apical shoot should be the first choice for use in the new method for assessment of RCR severity. The RFW of roots also showed a high correlation coefficient

Fig. 2. Impact of *Calonectria ilicicola* inoculum density (0.2–3.2%) on growth of soybean plants (cultivar Enrei) at 14 days post-inoculation (dpi). (A) Representative photograph of the whole plant and (B) relative fresh weight (RFW) of root, shoot, and apical shoot, and visual disease severity. Different letters indicate significant difference at the 5% level according to Tukey's Honest Significant Difference (HSD) Test ($p < 0.05$) test. Values are means $\pm$ SD, $n = 30$. There were three replications and each replicate consisted of 10 plants, $n = 30$.

Fig. 3. The correlation analysis between visual disease severity and relative fresh weight of shoot (blue square), root (grey triangle), and apical shoot (white circle) of Enrei.

($R^2 = 0.89$, $p < 0.05$); however, determination of root RFW is very laborious and time consuming, and so is less advantageous compared to the others.

Validation of fresh weight-based measurement in genetic differentiation of *C. ilicicola* resistance

Two experiments, each contains 27 and 17 genotypes, respectively, were conducted using 1.2% inoculum density to validate the RFW of apical shoot as an index of RCR severity. The results of one-way ANOVA indicated a significant genotypic variation ($p < 0.002$) in RFW of apical shoot and visual disease severity ($p < 0.000$). According to one-way ANOVA results, visual disease severity and RFW of apical shoot for the first and second validation experiments are presented in **Supplemental Tables 1** and **2**.

The validation experiment using 24 soybean cultivars together with the three wild soybean accessions (**Supplemental Table 1**) showed that the RFW of apical shoot was clearly different between *C. ilicicola* resistant wild soybean accessions (Jiang *et al.* 2020) and cultivated soybean cultivars (**Fig. 4**). Based on Tukey's Honest Significant Difference (HSD) Test ($p < 0.05$), the greatest values of RFW were detected for wild soybeans, while their visual disease severities were the lowest among the tested accessions. The *C. ilicicola*-infection caused significant changes in RFW of the soybean cultivars, whereas it was not the case for *C. ilicicola* resistant wild soybeans. Among the soybean cultivars, Nakasennari and Murayutaka exhibited relatively high RFW of apical shoot (≥ 0.45) and low disease severity (≤ 2.77) compared to the others. The correlation coefficient (R^2) of RFW-based measurement to visual disease severities was 0.72 ($p < 0.001$) (**Fig. 4**). However, in both visual

Fig. 4. The relationship between visual disease severity and relative fresh weight (RFW) of apical shoot of 24 soybean cultivars and three wild soybean accessions at 14 days post-inoculation (dpi) with *Calonectria ilicicola* (1.2%). The soybean cultivars and wild soybean accessions are shown in square filled with grey (Satonohohoeimi), black (LD00-3309), purple (Osuzu), brown (UA4805), white (Nemashirazu), red (Ayakogane), yellow (Gendenshirazu), blue (Tachinagaha), and green (Yukishizuka), circle filled with blue (Murayutaka), green (Nakasennari), red (Akimaro), black (Tamahomare), pink (Peking), grey (Shuurei), purple (Otsuru), and yellow (Yukihomare), triangle filled with green (wild 9), blue (wild 7), and purple (wild 27), and rhombus filled with blue (Nattosoryu), green (Miyagishirome), yellow (Nanbushirome), black (NC-Raleigh), brown (Enreinosora), grey (Ryuhou) and red (Enrei). There were three replicates per genotype, each replicate consisted 5 plants, $n = 15$.

Fig. 5. The relationship between visual disease severity and relative fresh weight (RFW) of apical shoot of 14 soybean cultivars and three wild soybean accessions following inoculation at 14 days post-inoculation (dpi) with *Calonectria ilicicola* (1.2%). The soybean cultivars and wild soybean accessions are shown in square filled with blue (Conrad), green (Himeshirazu), yellow (Williams 82), purple (Wabash), red (Enrei), and black (Shoutou 1 Chu), circle filled with yellow (Fukuminori), blue (Shoutou 1 EN), green (Amagi Zairai), red (Sachiyutaka), purple (Yukihime), black (Horokanai Zairai), white (Kitamishiro) and brown (Tanbakuro), triangle filled with green (wild 9), blue (wild 7), and purple (wild 27). There were three replicates per genotype, each replicate consisted 5 plants, $n = 15$.

and RFW methods, it appears difficult to determine minor differences in *C. ilicicola* resistance among the susceptible soybean cultivars.

Further validation using 14 soybean cultivars together with the three wild soybean accessions (**Supplemental Table 2**) gave similar results, which clearly distinguished the *C. ilicicola* resistant wild soybean accessions from the soybean cultivars. In addition, the cultivars Tanbakuro, Horokanai Zairai and Kitamishiro showed higher RFW of apical shoot (≥ 0.7) and lower visual disease severity scores (≤ 2.59) compared with the other soybean cultivars (**Fig. 5**, **Supplemental Table 2**). The correlation coefficients (R^2) of RFW-based measurement to visual disease severities were 0.79 ($p < 0.001$) (**Fig. 5**).

It should be noted that the degree of disease severity in both visual and RFW measurements can vary between experiments depending on pathogen growth, and environmental conditions like light and temperature. Therefore, the assessment of resistance between different cultivars should be done in the same experimental set. Moreover, a genetic variation for resistance to *C. ilicicola* was observed among soybean and wild soybean accessions, however, no complete resistance was detected; which is consistent with previous screenings for *C. ilicicola* resistance in large populations of soybean and wild soybean accessions (Jiang *et al.* 2020, Kim 1994, Nakajima *et al.* 1994).

In summary, we present a new method for the evaluation of *C. ilicicola* resistance that involves simply measuring the RFW of the apical shoot, which should greatly facilitate evaluation and screening for resistance to *C. ilicicola*, especially in large-scale population studies. This method showed

favorable consistency over the traditional visual scoring method for RCR severity, and efficiently differentiated *C. ilicicola* resistance among soybean and wild soybean accessions. The new method provides advantages over the traditional visual evaluation; these include simplicity, ease of performance, high uniformity and high sample throughput, and cost effectiveness. Whether this method is also applicable to field evaluation of *C. ilicicola* resistance remains unknown and deserves further investigation in the future studies.

Author Contribution Statement

KW performed experiments and wrote the manuscript; CJ conceived and designed the research, and revised the manuscript.

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Literature Cited

- Akamatsu, H., N. Fujii, T. Saito, A. Sayama, H. Matsuda, M. Kato, R. Kowada, Y. Yasuta, Y. Igarashi, H. Komori *et al.* (2020) Factors affecting red crown rot caused by *Calonectria ilicicola* in soybean cultivation. *J. Gen. Plant Pathol.* 86: 363–375.
- Bajgain, R., Y. Kawasaki, Y. Akamatsu, Y. Tanaka, H. Kawamura, K. Katsura and T. Shiraiwa (2015) Biomass production and yield of soybean grown under converted paddy fields with excess water during the early growth stage. *Field Crops Res.* 180: 221–227.
- Bani, M., D. Rubiales and N. Rispaill (2012) A detailed evaluation method to identify sources of quantitative resistance to *Fusarium oxysporum* f. sp. *pisi* race 2 within a *Pisum* spp. germplasm collection. *Plant Pathol.* 61: 532–542.
- Berggren, G.T. and J.P. Snow (1989) Red crown rot. *In:* Sinclair, J.B. and P.A. Backman (eds.) *Compendium of Soybean Diseases*, American Phytopathological Society. St. Paul, MN, USA, pp. 44–45.
- Bock, C.H., G.H. Poole, P.E. Parker and T.R. Gottwald (2010) Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *CRC Crit. Rev. Plant Sci.* 29: 59–107.
- Bock, C.H. and F.W. Nutter Jr. (2012) Detection and measurement of plant disease symptoms using visible-wavelength photography and image analysis. *Plant Sci. Rev.* 73.
- Crous, P.W., M.J. Wingfield and A.C. Alfenas (1993) *Cylindrocladium parasiticum* sp. nov., a new name for *C. crotalariae*. *Mycol. Res.* 97: 889–896.

- Gao, X., X. Lu, M. Wu, H. Zhang, R. Pan, J. Tian, S. Li and H. Liao (2012) Co-inoculation with rhizobia and AMF inhibited soybean red crown rot: from field study to plant defense-related gene expression analysis. *PLoS ONE* 7: e33977.
- Gao, X., M. Wu, R. Xu, X. Wang, R. Pan, H.-J. Kim and H. Liao (2014) Root interactions in a maize/soybean intercropping system control soybean soil-borne disease, red crown rot. *PLoS ONE* 9: e95031.
- Jackson, E.W., P. Fenn and P. Chen (2005) Inheritance of resistance to Phomopsis seed decay in soybean PI 80837 and MO/PSD-0259 (PI 562694). *Crop Sci.* 45: 2400–2404.
- Jiang, C.J., S. Sugano, A. Kaga, S.S. Lee, T. Sugimoto, M. Takahashi and M. Ishimoto (2016) Evaluation of resistance to *Phytophthora sojae* in soybean mini core collections using an improved assay system. *Phytopathology* 107: 216–223.
- Jiang, C.J., S. Sugano, S. Ochi, A. Kaga and M. Ishimoto (2020) Evaluation of *Glycine max* and *Glycine soja* for resistance to *Calonectria ilicicola*. *Agronomy* 10: 887.
- Kim, K.D. (1994) Susceptibility in soybean to red crown rot and characteristics of virulence in *Calonectria crotalariae*. Ph.D. Dissertation, Louisiana State University, Baton Rouge, Louisiana.
- Kim, K.D., J.S. Russin and J.P. Snow (1998) Effect of plant age on infection of soybean by *Calonectria ilicicola*. *Kor. J. Plant Pathol.* 14: 247–252.
- Kuruppu, P.U., R.W. Schneider and J.S. Russin (2004) Factors affecting soybean root colonization by *Calonectria ilicicola* and development of red crown rot following delayed planting. *Plant Dis.* 88: 613–619.
- MAFF (2014) Current situation and issues of soybean production in Japan (in Japanese). https://www.maff.go.jp/j/kokusai/kokkyo/yosan/attach/pdf/h28_jigyo_report-61.pdf.
- Nakajima, T., S. Sakai, T. Gomi and A. Kikuchi (1994) Development of methods for assessing resistance to black root rot caused by *Calonectria crotalariae* in soybean [*Glycine max*] and screening for resistant germplasm. *Bull. Tohoku Natl. Agric. Exp. Stn.* 88: 39–56 (in Japanese with English summary).
- Nishi, K., F. Sato, T. Karasawa, T. Sato, T. Fukuda and H. Takahashi (1999) Ecology and control of root necrosis of soybean caused by *Calonectria crotalariae*. *Bull. Nat. Agric. Res. Cent.* 30: 11–109 (in Japanese with English summary).
- Ochi, S., M. Yoshida, A. Nakagawa and M. Natsume (2011) Identification and activity of a phytotoxin produced by *Calonectria ilicicola*, the causal agent of soybean red crown rot. *Can. J. Plant Pathol.* 33: 347–354.
- Padgett, G.B., P.U. Kuruppu and J.S. Russin (2015) Red crown rot. In: Hartman, G.L., J.C. Rupe, E.J. Sikora, L.L. Domier, J.A. Davis and K.L. Steffey (eds.) *Compendium of soybean diseases and pest*, The American Phytopathological Society Press, St. Paul, MN, USA, pp. 79–80.
- Pilet-Nayel, M., F.J. Muehlbauer, R.J. McGee, J.M. Kraft, A. Baranger and C.J. Coyne (2002) Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. *Theor. Appl. Genet.* 106: 28–39.
- Raftoyannis, Y. and M.W. Dick (2002) Effects of inoculum density, plant age and temperature on disease severity caused by pythiaceae fungi on several plants. *Phytoparasitica* 30: 67–76.
- Roy, K.W., K.S. McLean, G.W. Lawrence, M.V. Patel and W.F. Moore (1989) First report of red crown rot on soybeans in Mississippi. *Plant Dis.* 73: 273.
- Shakiba, E., P. Chen, R. Gergerich, S. Li, D. Dombek, A. Shi and K. Brye (2012) Reactions of mid-southern US soybean cultivars to *Bean pod mottle virus* and *Tobacco ringspot virus*. *Crop Sci.* 52: 1980–1989.
- Sugimoto, T., M. Kato, S. Yoshida, I. Matsumoto, T. Kobayashi, A. Kaga, M. Hajika, R. Yamamoto, K. Watanabe, M. Aino *et al.* (2012) Pathogenic diversity of *Phytophthora sojae* and breeding strategies to develop *Phytophthora*-resistant soybeans. *Breed. Sci.* 61: 511–522.
- Yamamoto, R., A. Nakagawa, S. Shimada, S. Komatsu and S. Kanematsu (2017) Histopathology of red crown rot of soybean. *J. Gen. Plant Pathol.* 83: 23–32.