

Identification of Broomrape (*Orobanche Cumana* Wallr.) Biotypes in the Main Sunflower Growing Areas of China

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Abstract

In order to identify the distribution and race composition of sunflower broomrape (*Orobanche cumana* Wallr.), seed was collected from the main sunflower production areas including Inner Mongolia and Xinjiang autonomous regions, and Gansu and Heilongjiang Provinces, China in 2014, 2016 and 2017. Bulk seed samples from 55 areas collected in 2014 were evaluated against a set of differential lines. The biotypes of *O. Cumana* were Races D-F in Inner Mongolia, Races B-F in Xinjiang, predominantly Race F in Gansu, and Race E in Heilongjiang. Of the 44 areas collected in 2016, a wide distribution of broomrape Races F-G or higher was observed in Inner Mongolia, which had the highest number of biotypes, as well as the fastest spread. The distribution of broomrape biotypes varied in Xinjiang for Races D-F. Gansu and Heilongjiang were both predominantly Race E. The speed of change of Races D to F in Xinjiang accelerated in recent years. The sampling was not at the same site, but within the same general area, and our results should explain the current status of broomrape races in China. The evaluation of the 90 areas collected in 2017 is in progress. The evaluation of 16 representative broomrape samples in each of three growing areas was conducted. In Inner Mongolia, 11 of the 16 broomrape samples were Race F and the remaining were Races D-E. In Gansu, however, 3 of the 16 samples were Race D, and the others were Races E-F. In Xinjiang, the biotypes of all samples were Races C-F. The variability in biotypes observed in selected sampled areas represented the population dynamics of broomrape races in natural condition without the selection pressure of resistant sunflower hybrids.

Keywords: Sunflower, *Orobanche*, Broomrape

Introduction

Orobanche cumana is a non-photosynthetic root parasitic plant that specifically infects sunflower. It does not have roots, but only haustoria that absorb nutrition and water from the sunflower plant. The infected sunflower plants are seriously deprived of nutrition resulting in weaker plants with smaller head diameters resulting in up to an 80% yield loss. Since the discovery of Race F that attacked all the known resistance genes in the early 1980's, sunflower breeders have been extremely busy identifying newer races and their corresponding resistance genes, and closely monitoring their spread and distribution globally (Akhtouch et al. 2002, Fernandez et al. 2008; Skoric et al. 2010). The same general trend of race shifts is occurring in China, with growing areas seeing an increased presence of race F and beyond (Zhao 2014; Ma and Jan 2014; Shi et al. 2015). Considering that the Chinese sunflower production is predominantly confectionery, which lacks higher levels of broomrape resistance genes than oilseed, this rapid race shift is extremely alarming. Therefore, broomrape is listed as a quarantine requirement when importing sunflower seed into China. Currently, broomrape distribution includes Xinjiang, Gansu, Inner Mongolia, Shaanxi, and the eastern provinces of China. Monitoring the broomrape race composition in each area using bulk samples to identify the highest race(s) present will not only provide information about race dynamics and competition, but will also serve as guidelines for breeding resistant sunflower hybrids.

Materials and Methods

Broomrape seeds were collected from the main sunflower growing areas in 2014, 2016 and 2017. Bulk samples from 55 fields were collected in 2014. A total of 44 representative fields were sampled in 2016 by collecting seeds from 60 random broomrape plants from each field, including 30 fields in Inner Mongolia, one in Heilongjiang, 3 in Gansu, and 10 in Xinjiang. The 2016 collected bulk broomrape seed from each field was used to infect a set of differential lines resistant to broomrape Races A to F (71NCA101, J8281, 1532, S1358, NR5, and P96, respectively), together with the universal susceptible line B1117 to identify the highest race in each field. Of the fields sampled in 2017, 15-16 samples from one representative field from Inner Mongolia, Gansu, and Xinjiang were also evaluated for race composition of individual fields.

Small pots were prepared and filled with a mixture of soil, vermiculite and sand in a 2:1:1 ratio. About 30 mg of broomrape seeds were applied to the soil mixture for each pot. Three pots were inoculated for each area and each differential line. Sunflower seeds were planted in the small pots after germination and transplanted at the V4 stage to regular 6-inch diameter flower pots and grown under greenhouse conditions. Proper watering in the greenhouse is critical since over-watering will stunt broomrape plant growth. The scoring of the emerging broomrape plants started at the R6 stage.

Results

Identification of the highest broomrape race found in the major Chinese sunflower growing areas in 2014 and 2016 is shown in Table 1. In Inner Mongolia, the number of fields in 2014 having Races D, E, and F was 1, 2, and 16, respectively. However, in 2016 Races D and E disappeared and Race F became the predominant race in 26 fields, while Race G began to appear in 3 fields. Races B, C, D, E, and F were present in Xinjiang in 2014, but Races B and C disappeared in 2016, leaving only Races D, E, and F. In Gansu, Race F was present in 2 fields in 2014, but absent in 2016, while Race E was absent in 2014, but appeared in 3 fields in 2016. Three fields in Heilongjiang were identified with Race F in 2014 and 2 fields in 2016.

Inner Mongolia was a region with a relatively high number of broomrape races including Races D-F. Race F was the predominant biotype in 16 of the 19 sampled areas in 2014, and 26 of 29 in 2016. Race G has occurred in the last 3 years appearing in three fields in 2016. However, the frequency of Race G broomrape is still at a low level, but seed is being increased in the greenhouse for future testing.

This study clearly indicates a trend in the disappearance of lower races such as Races B and C, and the increase of higher Races D, E, and F. Overall, Race F is the predominant race in China, especially in the major sunflower production areas of Inner Mongolia.

Results of individual broomrape samples collected in 2017 from each of the three selected areas were tested against five differentials for Races A-F and summarized in Table 2. Among all samples from Inner Mongolia, 11 samples appeared to infect the NR5 differential (resistant to Race E in Spain), suggesting that a high frequency of Race F existed in the field, compared to 4 for Race E, and 1 for Race D in the same field. The four samples infected S1358 (resistant to Race D) by a low degree of infection equal to 5, 1, 3, and 2 confirmed the existence of Race E. The Race D infected 1532 (resistant to Race C) with 5 shoots per plant. In Gansu, 12 samples were Race E infecting S1358 (resistant to Race D). However, three samples had a small percentage of broomrape overcoming the *Or₃* resistance gene of sunflower in the 1532 differential line, suggesting the existence of Race D. Only one sample infected NR5, suggesting Race F. In Xinjiang, the biotypes of all the samples ranged from Races C-F. Four samples overcame S1358 (resistant to Race D), 4 samples infected NR5 (resistant to Race E), suggesting Races E and F, respectively. Other samples collected showed degrees of infection among the control J8281 (resistant to Race B), 1532 (resistant to Race C), indicating the presence of Races C and D, respectively.

Discussion

In 1959, the sunflower broomrape was first discovered in Zhaozhou County, Heilongjiang Province in China. For a long time after its discovery, broomrape was not a problem in sunflower cultivation and no damage or dispersal was reported. It came to the attention of the Chinese sunflower community only in recent years due to the drastically increased activities of importing and transporting sunflower seed, and lack of attention to plant quarantine. The presence and spread of broomrape to major sunflower production areas in Inner Mongolia, Xinjiang, Heilongjiang, Jilin, Liaoning, and Shaanxi could be caused by the continuous cropping of the same fields with seeds contaminated with higher level races.

In the largest and most concentrated area of sunflower cultivation, Bayannaer of Inner Mongolia, broomrape has infected up to 13,300 ha with a parasitic rate as high as 90% resulting in yield losses of 30 to 45%.

Currently, the planted acreage of sunflower in the main sunflower production area is about 1.3 million acres in 2017, but is expected to decrease to 1.1 to 1.2 million acres in 2018. The results in the current study were largely based on the natural coexistence of broomrape races in fields without selection pressure from growing resistant hybrids. The frequency of the different races in a single field also serves to indicate the relative competitiveness of races.

Comparing the results of 2014 and 2016, without any obvious selection pressure, a general trend moving toward higher levels of broomrape races was obvious. As time goes on, we would expect a drastic increase of Race G or higher races spreading in China as Races E and F resistant hybrids are developed and widely used in production.

Previous reports suggested an abundance of broomrape resistance genes in wild *Helianthus* species, especially the wild perennials (Jan et al. 1998, 1999, 2000, 2002). Efficient resistance screening and utilization of those wild germplasms will play a critical role in the future success of sunflower when battling this most damaging parasitic weed.

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Table 1. The highest broomrape races in major Chinese sunflower growing areas in 2014 and 2016.

Region	Year	Broomrape Races						
		Race A	Race B	Race C	Race D	Race E	Race F	Race G
Inner Mongolia	2014	0	0	0	1	2	16	0
	2016	0	0	0	0	0	26	3
Xinjiang	2014	0	2	5	8	11	5	0
	2016	0	0	0	1	3	6	0
Gansu	2014	0	0	0	0	0	2	0
	2016	0	0	0	0	3	0	0
Heilongjiang	2014	0	0	0	0	0	3	0
	2016	0	0	0	0	0	2	0

Table 2. The number of broomrape samples from Inner Mongolia, Gansu, and Xinjiang infecting the differential lines resistant to specific broomrape races in 2017.

Location	Differentials Resistant to Specific Broomrape Races						Total sample
	71NCA101 A	J8281 B	1532 C	S1358 D	NR5 E	P96 F	
Inner Mongolia	0	0	1	4	11	0	16
Gansu	0	0	3	12	1	0	16
Xinjiang	0	2	5	4	4	0	15