ASSESSMENT OF THE GENETIC DIVERSITY IN *AEGILOPS TAUSCHII* (COSS.) BY USING SSR MARKERS AND MORPHYSIOLOGICAL TRAITS

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Abstract. *Aegilops tauschii* Coss. (2n=2x=14, DD) is a problematic weed and has contributed in the D genome of common wheat. The genetic diversity among 40 Chinese populations were evaluated by microsatellites marker and morphysiological traits. Dry weight biomass showed variation while in the case of plant height high variation was found between populations. We determined 27 alleles by using eight primers with an average of 3.37 allele per locus. The maximum polymorphism information content (PIC) was 0.63 with an average of 0.20 and maximum allele frequency was 1.00 with an average of 0.88. Cluster analysis divided *Aegilops tauschii* into different groups, which showed obvious genetic difference between these populations. This study will be helpful for weed management and wheat crop breeding program.
Keywords: *Aegilops tauschii, molecular marker, genetic diversity, morphysiological trait, microsatellites*

Abbreviations: PIC: Polymorphism Information Content

Introduction

(*Aegilops tauschii* Coss.) (*Ae. tauschii*) has a troublesome effect on wheat crop growing areas of China (Dudnikov, 2000; Zhang et al., 2007). It is native to tropical Asia to temperate Asia and Europe (Wei et al., 2008). It has infested about 330,000 hectares (Zhang et al., 2007) of winter wheat in different provinces including Shanxi, Shandong, Henan, Shaanxi, Inner Mongolia, Jiangsu and Hebei in China. Moreover, due to insufficient prevention and limitation of control strategies, the spreading pace of this weed tremendously damage winter wheat productivity, particularly in the main wheat producing regions. *Ae. tauschii* distributed from the Mediterranean region; present in Syria, Russia, Kazakhstan, Afghanistan, Pakistan, Turkey, all the way to Iran and extending to the eastwards of Yili Valley of Xinjiang in China (Li, 2005). Iran and Yili valley of Xinjiang were usually familiar with the center of the origin of *Ae. tauschii*. *Ae. tauschii* is recognized in natural habitats for its world distribution, whereas subspecies (strangulate) of *Aegilops* is native to south-western Caspian Iran and Afghanistan (Ogbonnaya et al., 2005; Wang et al., 2013; Kalia et al., 2016). Some scientists also proposed that *Ae. tauschii* presented in the Yellow river region in China was introduced with common wheat through trade along the Silk Road (Li and Mo, 2004; Zhao, 2007).

Ae. tauschii involved in the origin of the hexaploid wheat. Moreover, crop breeding has resulted in genetic diversity among the hexaploid wheat and *Ae. tauschii* populations. On the base of the morphological parameter, decidedly less genetic differentiation in the D genome of the wheat crop exists (Zahra et al., 2010). Due to the

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genetic similarity of *Ae. tauschii* with wheat, it may have an essential role in wheat crop improvement (Knaggs et al., 2000). Through hybridization, many useful biotic and abiotic stress resistance genes of *Ae. tauschii* can be utilized in wheat variety improvement programs (Hsam et al., 2001).

Genetic diversity can assessed by phenotypic (physiological and morphological parameters) and microsatellite markers (AFLP, RFLP, and SSR). Morphological parameters of crops are useful for introductory assessment because of their rapid range for diversity (Majidi et al., 2009; Sun et al., 2014). Molecular markers used to evaluate the genetic diversity because of their polymorphism, reproducibility, co-dominance, and simplicity (Roder et al., 1995). In molecular markers, simple sequence repeats (SSR) are universal and commonly used for genetic diversity evaluation (Vieira et al., 2016). Furthermore, SSR markers are more critical for crop improvement (Mian et al., 2005). *Aegilops* species possessing the D genome could be rich abiotic sources. Molecular markers have been used in several studies to assess genetic diversity among different populations (Roy et al., 2006). Genes from *Ae. tauschii* can be utilized in wheat variety improvement programs (Hsam et al., 2001).

In China, *Ae. tauschii* is a problematic weed and competes for resources from the early stage until maturity stage in wheat. It is scattered in more than ten provinces all across the country. Due to morphological similarities with the wheat crop, it is difficult to be controlled with herbicides. It is known that DD genome of *Ae. tauschii* has a rich source of potential variability. For genetic diversity of *Ae. tauschii* populations collected from five different provinces of China were assessed by using morphysiological traits and fluorescent dye-labeled SSR markers. The results of this study will be beneficial to both weed management and wheat breeding.

Materials and methods

Forty populations of *Ae. tauschii* seeds were used from five different provinces (Henan, Shaanxi, Shandong, Hebei, and Shanxi) of China (*Table 1*, *Fig. 1*). The experiment was conducted at the greenhouse of the Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing, China. Each population was replicated four times following a completely randomized design for pot experiment. Plants floated over half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Sixty days after sowing, plant height (cm) and dry weight biomass (g) were recorded. For SSR analysis, plant leaves were taken and stored at -80ºC for DNA extraction. DNA was isolated from leaves by plant kit (TIANGEN). DNA purification and quantification were carried out following Shah et al. (2009). SSR primers were designed by BATCHPRIMER3 (*Table 2*).

Table 1. *Aegilops tauschii populations collected from different provinces of China*

Name of province	Population Collection
Henan	10
Shaanxi	3
Shandong	10
Hebei	12
Shanxi	5
Total	40

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Figure 1. *Collection of Aegilops tauschii population's seed from different provinces of China. Circles indicated provinces for seed collection*

Table 2. *Details of forward and reverse SSR primers*

Primers	Forward Primer (5'-3')	Reverse Primer (5'-3')
AG-5	AGAACATCTGGCGTAACATAG	GGTTTTGTCGCAGAATTAGTA
AG-7	AGCTTCATACGGCTTCTCTAT	AGCGCTTTTTCTTATTCTAGC
AG-12	TGCAGAAACTACCCAAATCTA	GCCACAAGGGACTATCTAAAC
AG-14	AGAGCAAATATAGGACCCAAG	CTCTCGTATTCGTCCTCTGA
AG-15	ATTATCGCTTAGCTTTCGACT	GTTGCAAAAATAAGAGCTTGA
AG-18	TTGACACGAGGAACTACTCAC	CTGTCTCGCAATACCTTCTAC
AG-19	CTTTGCCACCTACTGCTACTA	CGGATACTGCCATACAATTAC
AG-20	CCAGTTAAGGTGGGATATGAT	GATTGGCGGATTTCTAATAGT

Polymerase chain reactions (PCRs) were carried out in 20 μL reactions comprising 40 ng of genomic DNA 1 μL, 0.6 μM of each forward and reverse primer, 7.8 μM of ddH_2O and 10 μM of PCR master mix. The PCR profile consisted of denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 30 sec, 45°C-60°C (depending on primers) for 30 sec and 72°C for 2 min, with a final extension at 72°C for 10 min. The genetic diversity of these populations were analyzed by using eight SSR fluorescent markers by capillary electropherogram.

Statistical analysis

Means, variances and coefficient of variances for plant height and dry weight biomass were measured by Statistix-8.0. For molecular data, allelic size was determined by using GeneMarker version 2.2.0 (Applied Biosystems). PowerMarker 3.1 was used to determine the allele amplification, allele frequency and polymorphism information content. Phylogenetic tree was drawn by PowerMarker MEGA 3.5.

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Results

Means, variances and coefficient of variance in 40 *Ae. tauschii* populations showed variation in dry weight biomass and plant height (*Table 3*). Different populations have a different coefficient of variance in plant height, with maximum 12% and minimum 1.8%. In dry weight biomass, maximum and minimum coefficient of variance were recorded 25% and 3% in different populations. Analysis of variance in plant height and dry weight biomass showed a relationship between populations, but some populations showed variation as compared with others in these two traits. Populations 6, 9, and 23 showed variation in plant height (*Fig. 2*), and populations 23 and 26 showed variation in dry weight biomass (*Fig. 3*). Amplification of eight primers in *Ae. tauschii* showed 27 alleles with an average of 3.37, maximum polymorphism information content (PIC) was 0.63 with the average of 0.20, and allele frequency ranges 0.41 to 1 with an average of 0.88 (*Table 4*). D genome microsatellite markers revealed the allelic range in the Chinese *Ae. tauschii* populations and has extended and showed polymorphism.

Table 3. *Mean standard deviation (STDEV) and coefficient of variance (CV %) of plant height (cm) and dry weight biomass (g) of 40 Aegilops tauschii populations*

Populations	Plant Height (cm)			Dry Weight Biomass (g)		
	Mean	STDEV	CV%	Mean	STDEV	CV%
PI	11.7	0.75	8.5	3.94	0.29	7
P2	15.0	1.47	9.8	3.24	0.45	14
P3	17.9	1.03	5.8	3.29	0.38	11
P4	13.6	0.30	2.2	3.57	0.44	12
P5	14.6	0.43	2.9	3.51	0.53	15
P6	12.1	0.45	3.8	3.00	0.49	16
P7	13.8	0.57	4.1	3.50	0.41	12
P8	13.8	0.24	1.8	3.29	0.26	8
P9	13.9	0.63	4.5	3.87	0.12	3
P10	14.4	0.48	3.3	4.02	0.11	3
P11	14.3	0.65	4.5	2.85	0.25	9
P12	14.4	0.51	3.5	3.10	0.33	11
P13	13.8	0.65	4.7	2.71	0.38	14
P14	15.8	0.73	4.6	3.14	0.42	13
P15	13.8	0.74	5.4	3.07	0.38	13
P16	12.6	0.48	3.8	2.92	0.23	8
P17	11.6	0.75	6.5	2.91	0.16	6
P18	14.4	0.48	3.3	2.98	0.27	9
P19	10.9	1.07	9.8	2.80	0.08	3
P20	15.5	0.41	2.7	2.72	0.17	6
P21	15.3	0.39	2.6	3.38	0.31	9
P22	12.6	0.75	5.9	3.81	0.13	4
P23	13.4	0.48	3.6	5.02	0.50	10
P24	11.4	1.37	12.0	3.89	0.21	5
P25	11.7	0.53	4.5	3.13	0.13	4
P26	13.2	0.40	3.0	2.49	0.52	21
P27	12.6	0.43	3.5	2.69	0.30	11
P28	14.9	0.84	5.6	2.80	0.24	9
P29	13.2	0.29	2.2	3.78	0.23	6
P30	14.2	0.86	6.0	3.56	0.31	9
P31	14.9	0.52	3.5	3.91	0.38	10
P32	12.5	0.71	5.7	3.84	0.10	3
P33	13.1	0.59	4.5	3.82	0.66	17
P34	12.3	0.86	7.0	3.00	0.35	12
P35	15.4	0.48	3.1	3.46	0.88	25
P36	14.4	0.48	3.3	2.79	0.39	14
P37	14.2	0.26	1.8	3.17	0.46	15
P38	14.6	0.42	2.9	3.20	0.26	8
P39	12.2	0.93	7.6	2.94	0.34	12
P40	15.0	1.42	9.4	3.10	0.26	9

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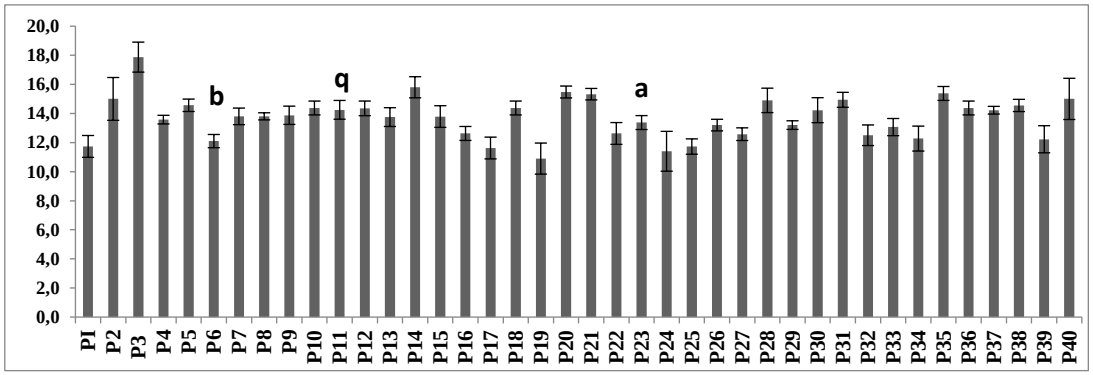

Figure 2. *Mean value of plant height of 40 populations of Aegilops tauschii. Bar indicating with letters showed variation. While other populations showed a close relationship with each other*

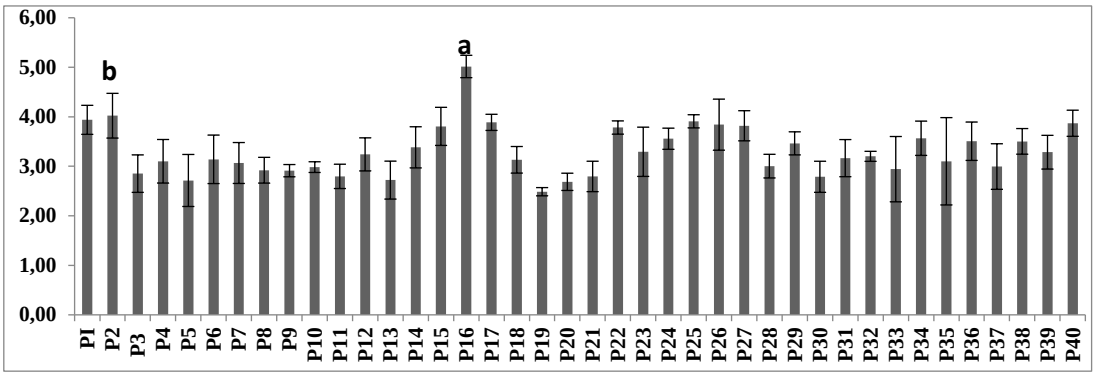

Figure 3. *Mean value of dry weight biomass of 40 populations of Aegilops tauschii. Bar indicating with letters showed variation. While other populations showed a close relationship with each other*

Table 4. *Allele amplification, allele frequency, and polymorphism information content for used SSR primers in 40 Aegilops tauschii populations*

Primers	Allele Amplification	Allele Frequency	PIC Value
AG-15	7	0.82	0.30
AG-7	4	0.41	0.63
AG-18	2	1.00	0.00
AG-14	4	1.00	0.53
AG-20	2	1.00	0.00
AG-19	4	0.87	0.21
AG-12	2	1.00	0.00
AG-5	2	1.00	0.00
Mean	3.37	0.88	0.20

On the base of cluster analysis (*Fig. 4*), 40 populations of *Ae. tauschii* were divided into three groups (I-III). Furthermore, the genetic relationships between populations elucidated by a dendrogram. Group I has six populations from Shanxi, Henan, and

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Shandong, showing their similar genetic background. Group II clusters have 26 populations of *Ae. tauschii* from Shaanxi, Shandong, Hebei, Henan, and Shandong provinces. The populations collected from Shaanxi, Henan, Shanxi, and Shandong were present in-group III. These results illustrated that the populations in the same group have a similar genetic background.

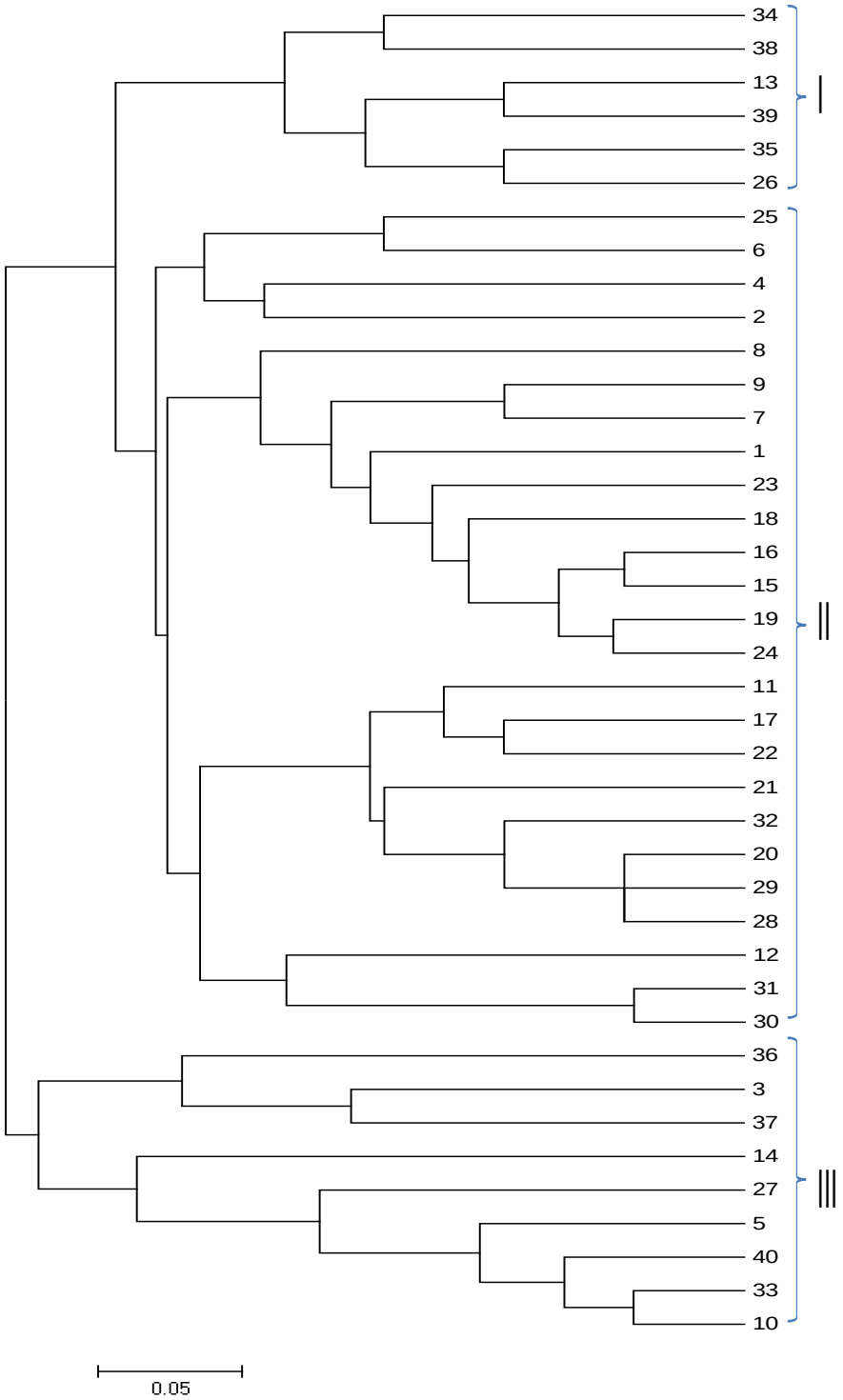

Figure 4. *Genetic relationship determined by PowerMarker (MEGA 3.5) cluster analysis based on the similarity coefficient of 40 populations of Ae. tauschii.*

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Discussions

The results showed the significant effect of *Ae. tauschii* on the genetic diversity by using morphysiological traits and SSR markers. Fifty-five populations of *Ae. tauschii* showed variances on the base of the morphysiological characteristics (Naghavi et al., 2007). Based on data recording plant height, some populations showed a close relationship with each other while populations 3 and 15 showed variation. Zara et al. (2010) and Ojaghi et al. (2010) reported high diversity in *Ae. tauschii* on the base of plant height, which is a little larger than ours. In our results, dry weight biomass showed close relationships between populations while in some populations showed variations. Zaharieva et al. (2003) reported higher genetic diversity in three species of *Aegilops* on the base of different morphological traits. Agronomic traits are available for wheat improvement and showed a high level of genetic diversity (Giuliani et al., 2009). Knaggs et al. (2000) reported that morphysiological characteristics of *Ae. tauschii* could be used in crop improvement varieties. Similarly, in the case of many studies, they believe that seed collection belongs to geographic regions in morphological diversity. Many studies showed that morphological and physiological parameters are not in compliance with the genetic diversity, while molecular markers cover a large part of the genome including coding and noncoding parts (Semagn, 2002). Our results showed that on the base of the molecular marker relationship between *Ae. tauschii* was obvious, while the genetic difference is present.

Genetic diversity based on morphological and physiological parameters were not confirming like SSR markers. No relationship was present between morphological and physiological parameters with genetic diversity (Tahernezhad et al., 2010). In microsatellite markers, simple sequence repeats (SSR) revealed higher genetic diversity in populations. A high level of polymorphisms and average allelic richness showed a high level of genetic diversity in crops. SSR markers also confirmed the phenotypic evaluation of populations (Zaharieva et al., 2003). The result on the base of eight SSR primers and morphological traits showed genetic diversity between populations. Obtained results were confirmed by Naghavi et al. (2007) with a PIC value of 9.21 and an allelic range of 6-15 that was achieved by the SSR marker and it could be helpful for a breeder in a crop breeding program. Results on the base of eight primers showed genetic diversity between populations. In the evaluation of the genetic diversity in Iranian *Ae. tauschii* populations using 13 microsatellites, a total of 66 alleles were amplified with PIC value of 0.65 (Saeidi et al., 2006). In our study, maximum (PIC) was 0.63 (*Table 4*) with the mean of 0.20, that was lower than 0.82 (Naghavi et al., 2007). Similarly, the genetic diversity of 46 *Commelina communis* populations using 12 SSR markers were assessed with an average PIC value of 0.20 (Yang et al., 2018). PIC values showed variation because they depended on (GT) content, number allele per locus, and type of motifs (Roder et al., 1995). Amplification of wheat SSR primers in *Ae. tauschii* (DD) showed similar regions between *Ae. tauschii* and wheat genome (Zhang et al., 2004). Microsatellite markers from the D genome revealed allelic amplification which showed polymorphism in *Ae. tauschii* populations of China. Allelic amplification showed a high level of genetic diversity in *Ae. tauschii* populations of Iran (Saeidi et al., 2006). This showed the relationship between populations and subdivisions in groups showed diversity. Amplification of universal wheat primers in *Ae. tauschii* of China showed the close relationship between wheat and *Ae. tauschii* (Pestsova et al., 2000).

Bibi et al. (2009) and Akhundova (2010) reported genetic variation among wheat populations using microsatellite markers with an allelic range of 2-4. SSR markers in

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wheat populations were reported with 3.6 alleles per primer (Ahmad, 2002; Almanza-Pinzon et al., 2003). Genetic diversity among wheat populations were studied with 21 SSR markers, which had an allelic range of 2-6, genetic similarity coefficient variation ranged from 0.45-0.90, and an average PIC value was 0.47 (Singh et al., 2006). Cluster analysis showed that populations from the same geographical region were present in the same group and their division in groups showed diversity. Populations from the same region have a great chance of being descended from a similar ancestor. On the basis of the genetic similarity coefficient, *Ae. tauschii* was divided into different groups. Cluster analysis divided *Ae. tauschii* (Iranian populations) into different groups and reported a high level of genetic variation between populations (Zahra et al., 2010).

Conclusion

Studying of *Ae. tauschii* populations of China by using molecular markers and morphysiological parameters has an essential role in wheat crop improvement. It is evident through molecular marker that *Ae. tauschii* will be helpful for crop breeding programs. Cluster analysis divided *Ae. tauschii* populations into three groups, which showed diversity and its different origins. High PIC value, allele amplifications and major allele frequency in some primers showed that specific parts of *Ae. tauschii* could be a valuable source for wheat crop improvement. Due to a rich source of potential variability, *Ae. tauschii* may perform vary when herbicides are applied in different wheat-growing areas. The results obtained from the study will help to weed management strategies in these regions.

Ethics and conflict of interests. The authors declare compliance with ethical standards and that they have no conflict of interests.

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