野生大豆和抗草甘膦转基因大豆杂交后代的适合度分析

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摘　要：野生大豆是大豆遗传改良的重要资源。转基因大豆可能对野生大豆资源存在潜在的农业和生态风险。外源基因从抗草甘膦转基因大豆向野生大豆材料的逃逸不仅需要成功的杂交，还要依赖于杂交后代的适合度。因此野生大豆和抗草甘膦转基因大豆杂交后代的适合度分析，对评价抗草甘膦转基因逃逸引起的生态风险非常必要。在网室条件下，4 个野生大豆材料和抗草甘膦转基因大豆 RR 能够杂交结实，获得有抗草甘膦基因杂交后代群体 F_1 和 F_2（江浦野生豆 −5 × RR）。对杂交后代及其母本野生大豆材料的 7 个农艺性状进行调查，计算适合度并进行 t 测验分析。结果表明：在没有草甘膦的选择压力下，杂交后代在一些性状上的相对适合度高于母本野生大豆材料；江浦野生豆 −5 和 RR 杂交 F_2 代敏感株与抗性株在 7 个农艺性状有相对适合度上均差异不显著。

关键词：野生大豆；抗草甘膦转基因大豆；杂交后代；适合度

中图分类号：S565. 1　　　　**文献标识码**：A　　　　**DOI**：10. 11861/j. issn. 1000-9841. 2015. 02. 0177

Fitness of Hybrids between Wild Soybeans（*Glycine soja*）and the Glyphosate-resistant Transgenic Soybean（*Glycine max*）

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Abstract：Wild soybeans are important resources for soybean genetic improvement. The potential agricultural and ecological risk of transgenic soybeans may pose a threat to wild soybeans. Transgene escape from glyphosate-resistant, transgenic soybeans to wild soybeans also lies on the fitness of hybrid offspring except for successful cross. Therefore, analysis on the fitness of hybrids between wild soybeans and glyphosate-resistant transgenic soybeans is essential to assess environmental consequences caused by glyphosate-resistant transgene escape. In this study, F_1 hybrids carrying the glyphosate resistance gene were obtained from crosses between four wild soybean and the glyphosate-resistant transgenic soybeans RR and reared under the net house conditions. Then, F_2 hybrids were derived from the cross between the wild soybean JPWS-5 and RR. Seven agronomic traits were measured in both hybrids and wild female soybeans. The relative fitness of the hybrids and wild females were analyzed by t-test. In the absence of glyphosate, we found that the hybrids between wild soybeans and RR had the relative fitness advantage of some traits to the wild female soybeans. The analysis on the fitness of F_2 hybrids between JPWS-5 and RR showed that there was no difference in the fitness for the seven agronomic traits between F_2 hybrids carrying the glyphosate resistance gene（F_2^+）and F_2 hybrids without the glyphosate resistance gene（F_2^-）. The results can provide scientific experimental data and theoretical support for safety assessment of transgenic soybeans and the protection of wild soybeans.

Keywords：Wild soybean；Glyphosate-resistant transgenic soybean；Hybrids；Fitness

Wild soybean（*G. soja* Sieb. and Zucc.）belongs to the subgenus *Soja* and is the immediate progenitor of the domesticated soybean（*G. max* L. Merr.）. Like the domesticated soybean, wild soybean is annual and diploid（2n = 40）, and these species are generally interfertile [1-5]. Wild soybeans frequently grow along roadsides, lakeshores, riverbanks, field margins and hedgerows, on mountain slopes and wastelands, in villages and sometimes the places adjacent to soybean fields [1, 3, 6-7]. Soybeans originated in China [1-5]. There are more than 6 000 accessions of wild soybean in China [1-4]. It has a particularly wide distribution, except for Qinghai, Xinjiang and Hainan province, extending from the north of Guangdong province in the south to the Amur River（Heilongjiang province）in the North of China [1, 3, 7-10]. Wild soybeans with richness of high yield, high quality, high resistance genes, and genetic diversity, are valuable resources for soy-

bean materials innovation and improvement breeding [1-2, 8]. Currently, wild soybeans are threatened by environmental pollution and various human activities, and the habitats have been erosed, isolated or fragmented [8, 11].

The glyphosate-resistant, transgenic soybeans were produced by introduction of the naturally glyphosate tolerant cp4 epsps coding sequence into the soybean genome using particle acceleration transformation [12-13]. Since 1996, when the Monsanto Company officially began promoting glyphosate-resistant, transgenic soybeans have become the most prevalent genetically modified (GM) crop in the world [14]. Some main countries of soybean production have planted the large-scale transgenic soybeans, however, the wild soybean is not native to those countries [9]. At present, although (GM) soybeans has not been approved for release in China, China imported large quantities of GM soybeans as processed raw materials every year [7, 9-11]. In addition, a longterm "National Transgenic Breeding Project" including soybean had been commenced since 2008 [11]. So, people are concerned about the possibility of gene escape from GM soybean varieties into the wild populations by hybridization [3-4, 7, 9-10]. Outcrossing between the wild and cultivated soybean was detected in some studies because the two species have no crossing barrier [1, 3-5, 13]. Oka reported that the highly fertile hybrids were obtained by artificial crossing. There was the introgression between the cultivated and wild soybeans in Japan and China [9, 15-16]. The weedy or semiwild form from the natural hybridization between the cultivated and wild soybeans had been reported [3, 7, 10, 13, 17]. Nakayama and Yamaguchi reported that the interspecific outcrossing rate varied from 0 to 5.89% with a mean value of 0.73%, when the wild and cultivated soybeans were planted together alternately in 5 × 12 arrays with 50 cm spacing on the farm[13]. Wang and Li collected 4 611 wild soybean seeds from three natural wild soybean populations within 200 cm close to the soybean fields, identified 31 hybrids and obtained 0.67% hybridization rate[7]. Therefore, wild soybeans and the transgenic soybeans could hybridize and produce seeds normally. Moreover, it was successfully reported by the natural hybridization [6, 18]. Mizuguti et al. planted glyphosate-tolerant GM soybean (AG3701RR) adjacent to wild soy-

bean, found their flowering periods overlapped 18-24 days and detected one hybrid out of 32502 [18]. Similarly, Mizuguti et al. obtained the maximum hybridization frequency (0.097%, 25/25741) from wild soybean crossed with GM soybean AG6702RR [6].

Transgene escape from glyphosate-resistant, transgenic soybeans depends first upon successful hybridization between wild soybeans and the transgenic soybeans, and second upon the fitness of hybrid offspring involving the exogenous genes[3-4, 19-20]. The above studies have examined the hybridization rates between glyphosate-resistant transgenic soybeans and wild soybeans [6, 18], however, so far, the fitness of such hybrids has not been reported. Fitness is defined as the proportionate contribution that an individual makes to the next generation. The relative fitness of hybrids was estimated the fitness of first and advanced-generation hybrids relative to the wild in other crops [19-21]. The objective of this study was to conduct artificial hybridizations between wild soybeans and the glyphosate-resistant transgenic soybean and to obtain hybrids. We further aimed to determine whether the potential hybrid plants survived and to what extent their fitness varied compared with the wild female soybeans without exposure to glyphosate. We also evaluate the fitness of those hybrids and speculate about the possibility that the glyphosate-resistant transgene will maintain in wild soybeans and diffuse through wild soybean populations. Then this research analyzed the relative fitness of the F_2 hybrids controlling genetic background. Thus these results are important to provide specific data to assess potential agricultural and ecological risk of the glyphosate-resistant transgenic soybean under no-glyphosate conditions.

Materials and Methods

Plant materials

In 2005, the wild soybean accessions ZYD4196, ZYD4152, ZYD4157, JPWS-5 and the glyphosate-resistant transgenic soybean accession RR were used in artificial hybridizations; F_1 hybrids of these crosses were obtained in 2006, and F_2 hybrids from the cross between JPWS-5 and RR were obtained in 2007. The wild soybeans were provided by the National Center for Soybean Improvement, Nanjing Agricultural University, and the glyphosate-resistant transgenic soybeans

were provided by the Monsanto Company, USA. An analysis of fitness was conducted using the F_1, F_2 hybrids and the wild female soybeans used to generate the crosses.

Experimental trials

Trials were carried out in the net house at Jiangpu Station ($32.12°$ N, $118.37°$ E), Nanjing, China from 2005 to 2007.

The wild soybeans ZYD4196, ZYD4152, ZYD4157 and JPWS-5 were sown on June 14, 2005, each wild soybean was bunch-planted in two rows, five bunches per row, four plants in each bunch, each row 400 cm-long and with 80 cm bunch and 100 cm row spacing. The paternal RR plants were sown on July 8, 2005, six rows, each row 200 cm-long and with 15 cm plant and 100 cm row spacing. There was 100 cm-wide corn buffer zone around the materials. The paternal RR plants, with white flowers, began to flower on August 16, while the maternal ZYD4196, ZYD4152, ZYD4157 and JPWS-5 plants, with purple flowers, began flowering on August 16, August 12, August 16 and August 14, respectively. The flowering periods of parent plants were matched. We subsequently performed artificial hybridizations, and the number of hybridized flowers and pods were counted. The F_1 and F_2 hybrids were single-planted with 10 plants per row, each row 400 cm-long and with 40 cm plant and 50 cm row spacing.

F_1 hybrids from the crosses between the wild soybeans and RR were identified by test strip[22] at the third or forth-true leaf stage in 2006. F_2 hybrids from the cross between JPWS-5 and RR were detected using Taqman probe technology via event-specific real-time quantitative PCR [23] in 2007. F_2 hybrids included F_2 hybrids carrying the glyphosate resistance gene (F_2^+) and F_2 hybrids without the glyphosate resistance gene (F_2^-). All primers and probes are listed in Table 1. The optimal PCR system was performed in a 20 μL reaction volume containing 10 μL of Platinum® Quantitative PCR SuperMix-UDG w/ROX (Invitrogen Company), 4 μL of 1 mmol·L^{-1} primer, 1 μL of 0.5 mmol·L^{-1} probe, and 5.0 μL of 15-20 ng·μL template DNA. The PCR reaction was performed on a ABI 7500 real-time PCR system with the following PCR parameters: 95℃ 10 min; followed by 40 cycles of 95℃, 15 s and 60℃, 60 s.

Table 1 Primer sequence, probe sequence and PCR products size of event-specific PCR

Name	Primer/probe	Primer sequence and probe sequence	Amplicon size/bp
NOS-Soy genome	Forward primer	CCTTTAGGATTTCAGCATCAGTGG	121
	Reverse primer	GACTTGTCGCCGGGAATG	
	Probe	5′-CGCAACCGCCCGCAAATCC-3′	
Lectin	Forward primer	GCCCTCTACTCCACCCCCA	118
	Reverse primer	GCCCATCTGCAAGCCTTTT	
	Probe	5′-AGCTTCGCCGCTTCCTTCAACTTCAC-3′	

Measurement and analysis of traits

Seven agronomic traits were measured in the wild female soybeans, F_1 and F_2 hybrids: plant height (PH), the main stem branch number (MSBN), shriveled pod number (SPN), number of pods per plant (PP), seed weight per plant (SWP), seed number per plant (SNP) and 100-seed weight (SW). To estimate relative fitness, the fitness of each characteristic was set as 1.00 in the wild female soybean. The relative fitness of F_1 and F_2 hybrids was then obtained as the ratio of the performance value of the hybrids to the value observed in the wild female soybean[21]. Differences in relative fitness between F_1, F_2 hybrids and the wild female parent as well as F_2^+ vs. F_2^- were analyzed using t-tests implemented in SAS software, version 9.0

(SAS Institute, Cary, NC, USA).

Results

Artificial hybridization

The wild and glyphosate-resistanttransgenic soybeans were hybridized and pods were obtained in 2005. The highest podding rate over all crosses (14.2%) was observed in hybrids of JPWS-5 and RR, while the lowest podding rate over all crosses (12.7%) was recorded in hybrids of ZYD4157 and RR in Table 2. The transgenic plant number of F_1 detected by test trip varied from 11 to 30, the proportion of glyphosate-resistant plants was 28.9%-60.0% in 2006. The F_1 plants carrying the glyphosate resistance gene were fertile.

Table 2　Artificial hybridization between four wild soybeans and RR in 2005

Traits of the F₁ hybrids	RR			
	ZYD4196	ZYD4152	ZYD4157	JPWS-5
Number of hybridized flowers	220	135	220	225
Pod number	30	18	28	32
Podding rate/%	13. 6	13. 3	12. 7	14. 2
Seed number	56	20	38	55
Plant number	33	12	22	42
Confirmation of hybrid status	test strip			
Transgenic plant number	26	12	11	30
Fructification of F₁	yes	yes	yes	yes

RR：the glyphosate-resistant transgenic soybean，was used in artificial hybridizations as the male parent；ZYD4196，ZYD4152，ZYD4157 and JPWS-5：wild soybeans，were used in artificial hybridizations as female parents；Pod number：the number of F₁ hybrids pods；Podding rate（%）= （Pod number/ Number of hybridized flowers）×100%；Seed number：the number of F₁ hybrids seeds；Plant number：the number of F₁ hybrids plants after emergence；Transgenic plant number：the number of F₁ hybrids plants carrying the glyphosate resistance gene detected by the test strip[22].

Fitness consequences in the absence of glyphosate

The fitness consequences for the F₁ hybrids in 2006 when no glyphosate selection pressure was applied are shown in Table 3. Four main patterns were observed. （1）The F₁ hybrids from the cross between ZYD4196 and RR exhibited higher relative fitness for PP and SNP compared to the maternal wild soybean ZYD4196；however, this difference was not significant （$P > 0.05$）. Additionally, the F₁ hybrids displayed a significantly higher relative fitness for PP （$P < 0.05$） and for MSBN, SPN, SWP and SW （$P < 0.01$） was significantly higher （$P < 0.01$） in the F₁ plants than in ZYD4196. （2）The F₁ hybrids from the cross between ZYD4152 and RR showed higher relative fitness for SNP compared to the maternal wild soybean ZYD4152, though this difference was not significant （$P > 0.05$）. However, the F₁ hybrids exhibited a significantly higher relative fitness for SWP （$P < 0.05$） and for SW （$P < 0.01$）, but significantly lower relative fitness for PH and MSBN （$P < 0.05$） compared to their female parent. The relative fitness for PP and SNP was significantly lower （$P < 0.01$） compared to ZYD4196. （3）F₁ hybrids from the cross between ZYD4157 and RR displayed lower relative fitness for PH than the maternal wild soybean ZYD4152, but this difference was not significant. The relative fitness for SWP and SW was significantly higher than in ZYD4152 （$P < 0.01$）. The relative fitness for MSBN, SPN, PP and SNP was also higher；however, these differences were not significant （$P > 0.05$）. （4）The relative fitness of F₁ hybrids from the cross between JPWS-5 and RR was significantly higher for SPN and SW （$P < 0.01$） compared to the maternal JPWS-5 plants, while the relative fitness for PH, PP and SNP was significantly lower （$P < 0.01$）. Finally, the F₁ hybrids showed higher relative fitness for SWP, lower relative fitness for MSBN compared to the maternal JPWS-5；however, these differences were not significant （$P > 0.05$）.

Table 3　Fitness of F₁ hybrids between four wild soybeans and RR and the wild female soybeans

Traits	Generation	RR			
		ZYD4196	ZYD4152	ZYD4157	JPWS-5
Plant number	F₁	26	12	11	30
	Female	8	6	9	11
PH/cm	F₁	146. 40 ± 14. 90ns (1. 31)	139. 92 ± 3. 19* (0. 81)	122. 40 ± 15. 39ns (0. 98)	157. 03 ± 5. 92** (0. 61)
	Female	111. 50 ± 17. 24 (1)	173. 17 ± 11. 57 (1)	124. 33 ± 10. 07 (1)	257. 64 ± 11. 80 (1)
MSBN	F₁	13. 24 ± 0. 79** (2. 82)	12. 08 ± 1. 05* (0. 52)	12. 00 ± 0. 82ns (1. 11)	11. 77 ± 0. 99ns (0. 78)

Table 3

Traits	Generation	RR			
		ZYD4196	ZYD4152	ZYD4157	JPWS-5
SPN	Female	4.69 ± 1.409 (1)	23.33 ± 3.38 (1)	10.78 ± 1.33 (1)	15.18 ± 1.29 (1)
	F_1	13.04 ± 5.45 ** (1.80)	3.25 ± 0.70 ns (1.50)	13.09 ± 6.97 ns (1.45)	8.30 ± 1.31 ** (3.25)
PP	Female	7.25 ± 1.69 (1)	2.17 ± 1.01 (1)	9.00 ± 3.18 (1)	2.55 ± 1.19 (1)
	F_1	305.62 ± 27.21 * (1.65)	367.92 ± 31.84 ** (0.61)	243.45 ± 41.84 ns (1.05)	240.73 ± 19.10 ** (0.44)
SNP	Female	185.75 ± 30.85 (1)	599.17 ± 74.33 (1)	232.00 ± 38.53 (1)	548.09 ± 83.58 (1)
	F_1	532.62 ± 47.70 ns (1.41)	525.08 ± 46.33 ** (0.40)	496.36 ± 88.31 ns (1.16)	462.43 ± 38.01 ** (0.40)
SWP/g	Female	378.38 ± 57.80 (1)	1306.00 ± 181.73 (1)	426.89 ± 82.99 (1)	1156.50 ± 181.92 (1)
	F_1	41.59 ± 4.39 ** (2.32)	28.54 ± 2.25 * (1.54)	23.50 ± 3.47 ** (2.30)	25.49 ± 2.35 ns (1.00)
SW/g	Female	17.92 ± 3.00 (1)	18.51 ± 3.05 (1)	10.21 ± 2.05 (1)	25.52 ± 4.22 (1)
	F_1	7.50 ± 0.24 ** (1.61)	5.53 ± 0.22 ** (3.92)	4.82 ± 0.19 ** (2.00)	5.48 ± 0.20 ** (2.50)
	Female	4.66 ± 0.28 (1)	1.41 ± 0.13 (1)	2.41 ± 0.16 (1)	2.19 ± 0.05 (1)

RR, the glyphosate-resistant transgenic soybean, was used in artificial hybridizations as the male parent; ZYD4196, ZYD4152, ZYD4157 and JP-WS-5, wild soybeans, were used in artificial hybridizations as female parents; Relative fitness is given in parentheses, the fitness of each characteristic was set as 1.00 in the wild female soybean, the relative fitness of F_1 hybrids was then obtained as the ratio of the performance value of the hybrids to the value observed in the wild female soybean [21]; * and ** represent significance at the 0.05 and 0.01 levels, respectively; ns, not significant; Plant number, number of the plants; PH, plant height; MSBN, the main stem branch number; SPN, shriveled pod number; PP, number of pods per plant; SWP, seed weight per plant; SNP, seed number per plant; SW, 100-seed weight.

F_2 population from the cross between JPWS-5 and RR were detected *via* real-time quantitative PCR in 2007. The plant number of F_2^+ was 232, and the plant number of F_2^- was 84 (Table 4). The results of the χ^2 test for glyphosate resistance in the F_2 population showed that the segregation for glyphosate resistance followed a 3:1 genetic ratio. It was consistent with previous results that the glyphosate resistance gene segregated in a simple Mendelian fashion [24-25].

The fitness consequences for F_2 hybrids derived from the cross between JPWS-5 and RR in 2007 are shown in Table 4. (1) The F_2 hybrids exhibited a higher relative fitness for SPN, SWP and SW ($P < 0.01$) and a lower relative fitness for PH ($P < 0.01$), SNP ($P < 0.05$) and PP (not significant, $P > 0.05$) compared with the maternal JPWS-5 plants. A similar result was observed in the comparisons between the maternal JP-WS-5 and both F_2^+ and F_2^-. F_2^- showed a significantly lower ($P < 0.05$) relative fitness for MSBN compared to JPWS-5. Similarly, the F_2 hybrids and F_2^+ displayed a lower relative fitness for MSBN, but the difference was not significant ($P > 0.05$). (2) In the comparison between F_2^+ and F_2^-, the relative fitness was higher for all traits, except for PH, for which there was no significant difference detected.

Table 4　Fitness of F_2 hybrids from the cross between JPWS-5 and RR

Traits	F_2^-	F_2^+	F_2	Female	t-test			
					F_2 vs. F	F_2^- vs. F	F_2^+ vs. F	F_2^- vs. F_2^+
Plant number	84	232	316	34				
PH/cm	141.49 ± 9.01 (0.65)	142.02 ± 4.28 (0.65)	141.88 ± 3.95 (0.65)	218.06 ± 7.23 (1)	* *	* *	* *	ns
MSBN	9.96 ± 0.38 (0.81)	10.39 ± 0.28 (0.85)	10.28 ± 0.23 (0.84)	12.24 ± 1.01 (1)	ns	*	ns	ns
SPN	10.92 ± 1.28 (2.32)	11.31 ± 0.75 (2.41)	11.21 ± 0.65 (2.40)	4.68 ± 0.95 (1)	* *	* *	* *	ns
PP	244.01 ± 16.00 (0.86)	256.01 ± 10.04 (0.90)	252.82 ± 8.50 (0.89)	284.41 ± 27.37 (1)	ns	ns	ns	ns
SNP	470.80 ± 35.30 (0.77)	486.58 ± 20.23 (0.79)	482.39 ± 17.54 (0.79)	614.32 ± 61.74 (1)	*	*	*	ns
SWP/g	27.40 ± 1.65 (1.45)	29.09 ± 1.08 (1.54)	28.64 ± 0.91 (1.52)	18.86 ± 1.68 (1)	* *	* *	* *	ns
SW/g	6.39 ± 0.16 (1.98)	6.52 ± 0.12 (2.02)	6.48 ± 0.10 (2.01)	3.23 ± 0.09 (1)	* *	* *	* *	ns

Relative fitness is shown in parentheses, the fitness of each characteristic was set as 1.00 in the wild female soybean, the relative fitness of F_2 hybrids was then obtained as the ratio of the performance value of the hybrids to the value observed in the wild female soybean [21]; * and * * represent significance at the 0.05 and 0.01 levels, respectively; ns, not significant; F_2 denotes F_2 hybrids from the cross between JPWS-5 and RR; F_2^- denotes F_2 hybrids without the glyphosate resistance gene; F_2^+ denotes F_2 hybrids carrying the glyphosate resistance gene; F, Female; Plant number, number of the plants; PH, plant height; MSBN, the main stem branch number; SPN, shriveled pod number; PP, number of pods per plant; SWP, seed weight per plant; SNP, seed number per plant; SW, 100-seed weight.

Conclusion and Discussion

Transgene escape from glyphosate-resistant, transgenic soybeans to wild soybeans also lies on the fitness of hybrid offspring except for successful cross[19-21]. The results showed that both higher and lower relative fitness values were obtained for seven agronomic traits in the F_1 and F_2 hybrids compared to wild soybeans when there was no glyphosate selection pressure in this study. Somewhat similar results regarding relative fitness have been reported for rape[26-27], rice [21], sunflower [28] and squash [20, 29]. Relative fitness is used to study the variation in fitness between wild relatives and their hybrid or backcrossed progeny, and speculate the possibility of the retention and diffusion of crop genes, including transgenic genes [21, 27-28, 30]. Therefore, there are many factors that may influence the changes in fitness observed in the hybrid and backcrossed progeny of transgenic crops and their wild relatives [19-20]. For example, research materials may not be original materials, and it may not be possible to distinguish between the effect of the presence of a transgene versus insertions, somatic mutations or other processes [20, 26]. Additionally, the introgression of crop genes other than the glyphosate-resistant gene into wild relatives could affect offspring fitness, and finally, there may be genetic obstacles to interspecific hybridization between crops and their wild relatives, leading to declines in fitness, although fitness might be restored in subsequent generations[26]. In this experiment, the podding rate of the hybrids was found to be as high as 14.2%, and the SPN of the hybrids was significantly greater than that of the maternal wild soybean. The relative SPN fitness calculated for the F_1 hybrids ranged from 1.45 to 3.25, and the fitness of F_2 hybrids derived from the cross between JPWS-5 and RR was 2.40. The nonsignificant difference of SPN between F_2^+ and F_2^- was mainly due to the fact that wild soybeans and cultivated soybeans are different species, and there may be hybridization incompatibility. Incompatibilities could result in abnormal meiosis of hybrid offspring, followed

by chromosomal translocation and pollen or egg cell a-bortion [1, 31]. Moreover, the relative fertility of the hybrids was calculated as the ratio of the average seed number in hybrids to that in wild plants [29]. This ratio was 0.44 for F_1 hybrids from the cross between JPWS-5 and RR, whereas the value for F_2 hybrids from this cross was as high as 0.79 in this study, which is consistent with previous results [26, 30]. Another factor affecting fitness is potential heterosis in crop-wild relative hybrids that increases offspring fitness [21]. In this study, the hybrids displayed certain advantages in reproductive traits (such as SWP and SW), which could be due to heterosis. The fitness of hybrids may also be affected by environmental conditions, the genotypes of the parents and genotype × environment interactions [19-20]. So, additional analysis should be conducted to examine the fitness of multiple hybrids from crosses between wild and transgenic soybeans, or their receptor materials, under the same conditions to understand the effects of transgenes and the possibility of diffusion within wild populations. Based on the forgoing discussion, not all of the changes in fitness may be due directly to the effects of transgenes, which was suggested by no difference for the fitness between F_2^+ and F_2^- in this study.

However, the hybrids between the wild and glyphosate-resistant transgenic soybeans had the relative fitness advantage of some traits to the wild female soybeans in the absence of glyphosate in this research. Moreover, Wang et al [25] reported transgenic F_2 hybrids had greater 5-enolpyruvoylshikimate-3-phosphate synthase (EPSPS) protein levels, per cent seed germination and more seeds per plant than nontransgenic controls, and suggested over-expression of transgene can cause fitness advantage even no glyphosate. Furthermore, the transgene will confer a selective advantage if the fitness of the wild populations is affected by the certain selective pressure on the transgene [19-20, 28]. Hence, wild soybeans may become more aggressive and finally caused further unwanted ecologic hazard, if the fitness of the wild populations with transgenes was largely increased. In addition, the wild soybeans can migrate through pollen dispersal [3, 8], seed ejection [11, 16] and long-distance dispersal caused by water, animals, birds or human activities [11, 32-33], then gradual-ly get closer to the farmland, thereby enhancing the risk of transgene escape [11]. And the researches reported that the natural hybridization between GM and non GM soybean could be controlled by spatial and temporal isolation [6, 18]. Therefore, if the GM soybeans would be released in China, an appropriate isolation between GM and wild soybeans should be strictly managed to avoid the possibility of transgenes diffusing in the wild population.

References

[1] Zhuang B C. Biological studies of wild soybeans in China [M]. Beijing：Science Press, 1999.

[2] Dong Y S, Zhuang B C, Zhao L M, et al. The genetic diversity of annual wild soybeans grown in China [J]. Theoretical and Applied Genetics, 2001, 103：98-103.

[3] Lu B R. Conserving biodiversity of soybean gene pool in the biotechnology era [J]. Plant Species Biology, 2004, 19：115-125.

[4] Lu B R. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In：Crop ferality and volunteerism [M]. Edited by Gressel J B. CRC Press, Taylor and Francis, Boca Rato, Florida, 2005：137-147.

[5] Andersson M S, de Vicente M C. Gene flow between crops and their wild relatives [M]. Foreword by NC Ellstrand. Johns Hopkins University Press, 2010：465-481.

[6] Mizuguti A, Ohigashi K, Yoshimura Y, et al. Hybridization between GM soybean (*Glycine max* (L.) Merr.) and wild soybean (*Glycine soja* Sieb. et Zucc.) under field conditions in Japan [J]. Environmental Biosafety Research, 2010, 9：13-23.

[7] Wang K J, Li X H. Interspecific gene flow and the origin of semi-wild soybean revealed by capturing the natural occurrence of introgression between wild and cultivated soybean populations [J]. Plant Breeding, 2011, 130：117-127.

[8] Jin Y, He T H, Lu B R. Fine scale genetic structure in a wild soybean (*Glycine soja*) population and the implications for conservation [J]. New Phytologist, 2003, 159：513-519.

[9] Wang K J, Li X H, Zhang J J, et al. Natural introgression from cultivated soybean (*Glycine max*) into wild soybean (*Glycine soja*) with the implications for origin of populations of semi-wild type and for biosafety of wild species in China [J]. Genetic Resources and Crop Evolution, 2010, 57：747-761.

[10] Wang K J, Li X H. Genetic diversity and gene flow dynamics revealed in the rare mixed populations of wild soybean (*Glycine soja*) and semi-wild type (*Glycine gracilis*) in China [J]. Genetic Resources and Crop Evolution, 2013, 60：2303-2318.

[11] Wang K J, Li X H. Genetic characterization and gene flow in different geographical-distance neighbouring natural populations of wild soybean (*Glycine soja* Sieb. & Zucc.) and implications for protection from GM soybeans [J]. Euphytica, 2012, 186：

817-830.

[12] Padgette S R, Re D B, Barry G F, et al. New weed control opportunities: development of soybeans with a Roundup Readpgene [M]//Dukeso. Herbicide Resistant Crops. Boca Raton, FL: CRC Press, 1996: 53-84.

[13] Nakayama Y, Yamaguchi H. Natural hybridization in wild soybean (*Glycine max* ssp. soja) by pollen flow from cultivated soybean (*Glycine max* ssp. max) in a designed population [J]. Weed Biology and Management, 2002, 2: 25-30.

[14] James C. Global status of commercialized Biotech/G M Crops: 2012. ISAAA Brief No. 44. ISAAA Ithaca NY.

[15] Abe J, Hasegawa A, Fukushi H, et al. Introgression between wild and cultivated soybeans of Japan revealed by RFLP anaysis for chloroplast DNAs [J]. Economic Botany, 1999, 53: 285-291.

[16] Oka H I. Genetic control of regenerating success in semi-natural conditions observed among lines derived from a cultivated × wild soybean hybrid [J]. Journal of Applied Ecology, 1983, 20: 937-949.

[17] Wang K J, Li X H, Li F S. Fine-scale phylogenetic structure and major events in the history of the current wild soybean (*Glycine soja*) and axonomic assignment of semi-wild type (*Glycine gracilis* Skvortz.) within the Chinese subgenus *soja* [J]. Journal of Heredity, 2012, 103: 13-27.

[18] Mizuguti A, Yoshimura Y, Matsuo K. Flowering phenologies and natural hybridization of genetically modified and wild soybeans under field conditions [J]. Weed Biology and Management, 2009, 9: 93-96.

[19] Jenczewski E, Ronfort J, Chèvre A M. Crop-to-wild gene flow, introgression and possible fitness effects of transgenes [J]. Environmental Biosafety Research, 2003, 2(1): 9-24.

[20] Hails R S, Morley K. Genes invading new populations: a risk assessment perspective [J]. Trends in Ecology and Evolution, 2005, 20 (5): 245-252.

[21] Song Z P, Lu B R, Wang B, et al. Fitness estimation through performance comparison of F_1 hybrids with their parental species *Oryza rufipogon* and *O. sativa* [J]. Annals of Botany, 2004, 93: 311-316.

[22] van den Bulcke M, de Schrijver A, de Bernardi D, et al. Detection of genetically modified plant products by protein strip testing: an evaluation of real-life samples [J]. European Food Research and Technology, 2007, 225(1): 49-57.

[23] Berdal K G, Holst-Jensen A. Roundup Ready soybean event-specific real-time quantitative PCR assay and estimation of the practical detection and quantification limits in GMO analyses [J]. European Food Research and Technology, 2001, 213: 432-438.

[24] Zhang N Y, Linscombem S, Oard J. Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice [J]. Euphytica, 2003, 130: 35-45.

[25] Wang W, Xia H, Yang X, et al. A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (*Oryza sativa*) without herbicide [J]. New Phytologist, 2014, 202 (2): 679-688.

[26] Snow A A, Andersen B, Jorgensen R B. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy B. *rapa* [J]. Molecular Ecology, 1999, 8: 605-615.

[27] Allainguillaume J, Alexander M, Bullock J M, et al. Fitness of hybrids between rapeseed (*Brassica napus*) and wild Brassica rapa in natural habitats [J]. Molecular Ecology, 2006, 15: 1175-1184.

[28] Snow A A, Moran-Palma P, Rieseberg L H, et al. Fecundity, phenology, and seed dormancy of F_1 wild-crop hybrids in sunflower (*Helianthus annuus*, Asteraceae) [J]. American Journal of Botany, 1998, 85: 794-801.

[29] Spencer L J, Snow A A. Fecundity of transgenic wild-crop hybrids of *Cucurbita pepo* (Cucurbitaceae): implications for crop-to-wild gene flow [J]. Heredity, 2001, 86: 694-702.

[30] Campbell L G, Snow A A, Ridley C E. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment [J]. Ecology Letters, 2006, 9: 1198-1209.

[31] Ahmad Q N, Britten E J, Byth D E. Inversion heterozygosity in the hybrid soybean × *Glycine soja* Evidence from a pachytene loop configuration and other meiotic irregularities [J]. Journal of Heredity, 1979, 70(6): 358-364.

[32] Cain M L, Milligan B C, Sterand A E. Long-distance seed dispersal in plant populations [J]. American Journal of Botany, 2000, 87: 1217-1227.

[33] Kuroda Y, Kaga A, Tomooka N, et al. Gene flow and genetic structure of wild soybean (*Glycine soja*) in Japan [J]. Crop Science, 2008, 48: 1071-1079.