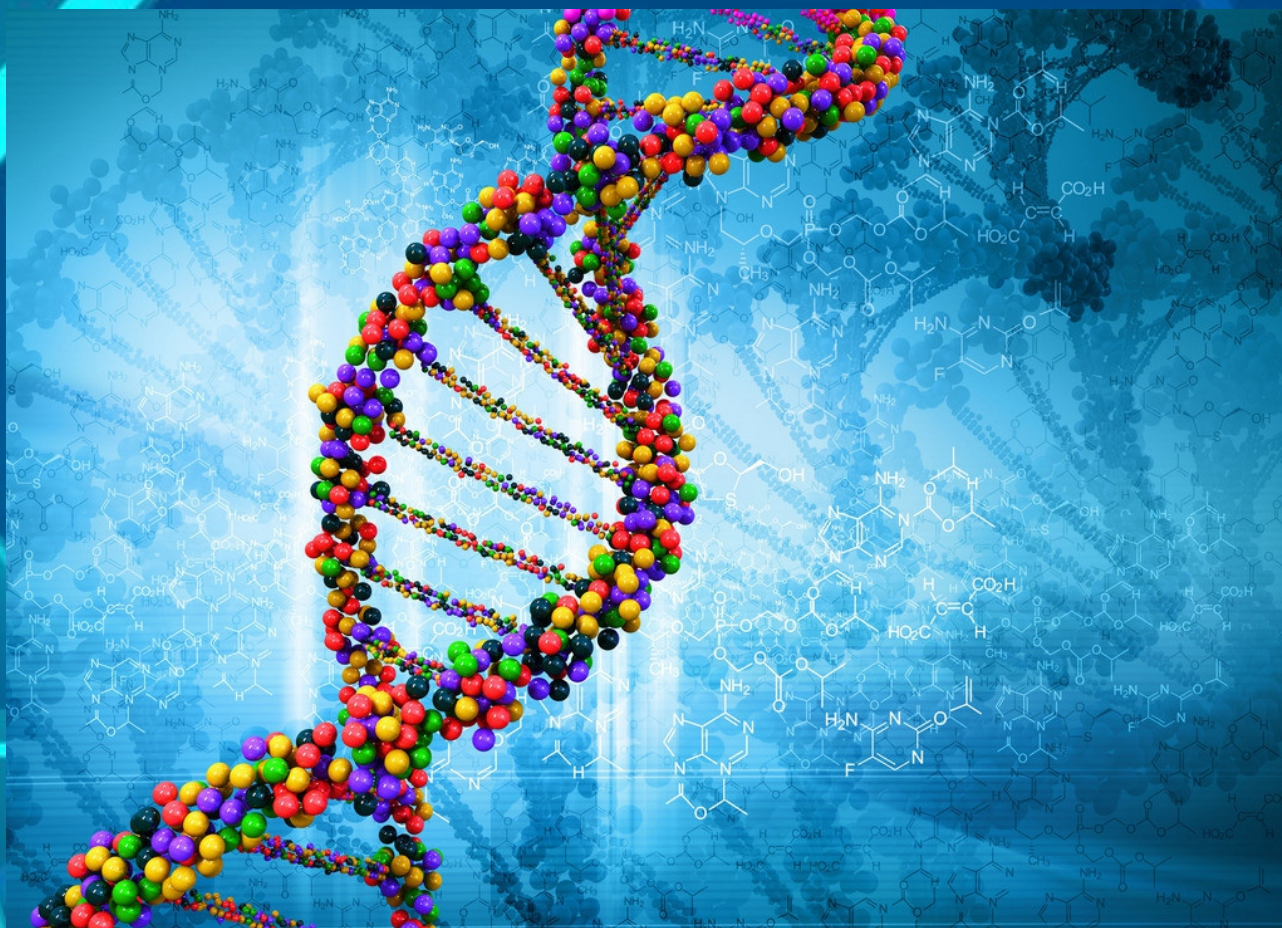


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Table of Contents

Volume 10 Number 2

June 2020

Validation of Competitive Ability of Diverse Canola Accessions against Annual Ryegrass under Glasshouse and Field Conditions

N. Shamaya, H. Raman, M. Rohan, J. Pratley, H. W. Wu.....17

Phenotypic, Cytological and Molecular (AFLP) Analyses of the Cotton Synthetic Allohexaploid Hybrid (*G. hirsutum* × *G. longicalyx*)²

N. O. Konan, J.-M. Jacquemin, J.-P. Baudoin, G. Mergeai.....35

Open Journal of Genetics (OJGen)

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Validation of Competitive Ability of Diverse Canola Accessions against Annual Ryegrass under Glasshouse and Field Conditions

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Abstract

Weeds are a major constraint in canola (*Brassica napus* L.) production worldwide, as they cause significant reductions in seed yield and quality. Crop interference is one of the approaches to tackle weed infestation along with other agronomic interventions. In Australia, studies have shown genetic variation in the canola capability to suppress annual ryegrass (*Lolium rigidum* Gaudin) in the field and under *in vitro* conditions. Early-season crop biomass accumulation and greater plant height are desired attributes for suppression weeds in canola. However, the canola ideotype for interference traits against this weed has not been studied under glasshouse conditions. In this study, we compared the competitive ability of 26 canola genotypes against annual ryegrass under both glasshouse and field conditions. Five canola genotypes consistently showed the ability to suppress growth of annual ryegrass. Both at glasshouse and field conditions, the shoot biomass, largely contributed by leaf biomass, was significantly associated with suppression ability. Our results suggest that a glasshouse-based evaluation approach can be used to determine the suppressive ability of advanced breeding lines for suppression of ryegrass growth. Based on our analysis, we suggest that initial screening of large collections of germplasm can be conducted under glasshouse conditions, with selected genotypes further evaluated in the field.

Keywords

Brassica napus, Weed-Crop Competition, Crop Interference, Ideotype

1. Introduction

Canola (*Brassica napus* L.) is an important oilseed crop grown worldwide. In

recent years, canola cultivation has expanded rapidly due to its high grain prices and demand for healthy vegetable oil, stockfeed and biodiesel markets [1]. Weed infestation, however, is a major constraint limiting canola production [2]. In Australia, total yield loss due to weeds in canola and pulses has been estimated at 122,048 tonnes, resulting in a revenue loss of \$54 million [3]. Annual ryegrass (*Lolium rigidum* Gaudin) has been the most widespread weed in winter crops, occurring in 86% of canola crops in south-eastern Australia [2].

The primary method of weed control is the application of herbicides. However, the prolonged and widespread chemical use has been increased especially after the introduction of herbicide-tolerant cultivars to triazine, imidazolinone and glyphosate. This has led to an increase in the evolution of herbicide resistance [4]. Canola seems to be particularly vulnerable for developing to herbicide resistance as there are limited options of commercial herbicide available to control broad-leaf weeds. In recent years, the canola industry is increasingly becoming dependent on herbicide tolerant varieties including genetically modified herbicide tolerant varieties, which are meant to provide control options for the major weeds of that crop, such as annual ryegrass and wild radish (*Raphanus raphanistrum*). Evaluation of the herbicides against different weed species showed that 8 of the top 15 are likely to be utilised in canola production, including imazamox and imazethapyr for the Clearfield® HR canola, glyphosate for the Roundup Ready® canola, and atrazine and simazine for the triazine tolerant lines are resistant to herbicides [5]. In recent years, some countries are imposing restriction on the usage of certain herbicides such as glyphosate for weed control; this practice necessitates the development of alternative and sustainable options for weed management. In addition to agronomic interventions that can influence weed management including seeding rate, row spacing, row orientation and fertilizer [6] [7], crop interference is worth investigating as a tool for weed management [8]-[14]. Considerable genotypic variation for weed competition exists in crop plants including canola, although some species are considered more competitive than others [15] [16] [17] [18]. For example, studies have shown canola to be less competitive on weeds than wheat and barley [19] [20] [21]. Vigorous hybrid canola varieties were found to be more competitive than open-pollinated varieties, largely attributed to greater hybrid vigour traits such as plant height and early-season crop biomass accumulation [9] [22] [23] [24] [25]. The competitive ability of a crop to weeds can be measured either on the basis of the ability of crop to maintain growth and seed yield in the presence of weed, or on the basis of the ability of crop to suppress growth and seed production of weed species [26]. Weed competitiveness in canola has been evaluated mainly under laboratory and field conditions and to a limited extent under glasshouse conditions [9] [27] [28]. Under field conditions, it is difficult to achieve precise and uniform plant densities across a trial site, and this may influence the differential responses obtained [26] [29]. Secondly, field conditions can compromise the outcomes through environmental variance [9] [26] [30]. Lastly, screening large

numbers of genotypes for weed-crop competition under field conditions is labour and space intensive [31].

The objective of this study is to evaluate the suppression ability of different canola genotypes against ryegrass. Obtaining reliable estimates of weed competitive ability and understanding the canola ideotype for interference traits are important for designing sustainable weed control strategies with low herbicide use for improving canola productivity and profitability.

2. Materials and Methods

2.1. Canola Genotype and Weed Populations

Previously, Asaduzzaman *et al.* [27] utilised 70 genotypes of canola to investigate genotypic variation for allelopathy and weed competitiveness. In this study, a set of 26 diverse *Brassica* genotypes (Table 1), including a subset of canola genotypes utilised by Asaduzzaman *et al.* [8] was characterised for their competitive ability against annual ryegrass cv. Wimmera under glasshouse and field conditions. This rye grass cultivar is well-suited to dry and low fertile soils and extensively used for productive, nutritious pasture crop. Seeds of canola genotypes were accessed from the National Brassica Germplasm Improvement Program (Wagga Wagga, Australia).

2.2. Evaluation of Canola-Weed Suppressive Ability under Glasshouse Conditions

The glasshouse experiment was conducted at the Wagga Wagga Agricultural Institute, NSW, Australia. The experiment was arranged in split-plot design with three replicates; main plots were 26 canola genotypes and subplots were the weed and weed-free treatments. Each experimental unit had four pots. The target canola density was five plants per pot in each treatment and ten annual ryegrass plants per pot in the weedy treatment. The 200 millimetre in diameter pots were filled with commercial garden mix containing: compost, manure, pine fines, sand and loam (3:3:2:1:1: by volume, 2 kg/pot). Fertiliser, 50 mL·pot⁻¹ (22.23 mL·L⁻¹ of water) of Thrive™ (NPK = 25:5:8.8), was added once per week. When canola plants started flowering, magnesium sulphate (2 g·L⁻¹ of water) was added once per week. Imidacloprid (15 g·L⁻¹) was used against aphids as needed, and Prothioconazole and Tebuconazole (210 g·L⁻¹) were used to control downy mildew disease as needed. The experiment was conducted under a 25/15°C day/night temperature regime with a 16-h photoperiod. Pots were watered daily to field capacity, avoiding any confounding effect due to moisture stress. At 50 d after sowing, shoots of canola were cut at the soil level to estimate stem and leaf biomass. Numbers of leaves on the main stem were counted and then leaves were separated from the stem and placed in separate paper bags. Both leaves and stems were dried at 70°C for 48 h, and weighed. The shoots of 10 annual ryegrass plants were harvested from each pot, dried at 70°C for 48 h and weighed. Plant height was measured from the soil level to the top of the

Table 1. Phenology and country of origin of canola accessions.

Genotype	Phenology	Country of origin
PAK85388-502	Semi-winter	Pakistan
X6-06-3275-3	Semi-winter	China
Ningyou7	Semi-winter	China
Av-Opal	Spring	Australia
Barossa	Spring	Australia
ATR-409	Spring	Australia
Sturt-TT	Spring	Australia
Hurricane	Spring	Australia
Av-Garnet	Spring	Australia
CB-Argyle	Spring	Australia
RP04	Spring	Australia
Ag-Outback	Spring	Australia
Skipton	Spring	Australia
Ag-Spectrum	Spring	Australia
BLN3343C001401	Spring	Australia
Rainbow	Spring	Australia
Rivette	Spring	Australia
ROY98310	Spring	Australia
Tarcoola-141	Spring	Australia
Lantern	Spring	Australia
CB-Trigold	Spring	Australia
Gross-Luesewitzer	Winter	Germany
Tapidor	Winter	Germany/France
Licapo	Winter	Germany
Beluga	Winter	Italy
Akela	Winter	Germany

canopy of the main (primary) stem.

2.3. Evaluation of Canola-Weed Suppressive Ability in the Field

The field experiments were conducted at the Wagga Wagga Agricultural Institute, Australia (35°30'07"S; 147°21'06"E) in a duplex Red Kandosol of pH 5.3. The field had a history of naturally high annual ryegrass population. Herbicides including glyphosate (450 g·L⁻¹) and oxyfluorfen (450 g·L⁻¹) were used to control annual ryegrass before sowing. Canola was sown on June 1, 2017 (due to unavailability of sufficient soil moisture during the main canola growing season i.e. late April to mid-May) with sowing rate of 1400 seeds plot⁻¹ and with 120

kg·ha⁻¹ of Croplift™ fertiliser treated with fungicide (Flutriafol, 500 g·L⁻¹). Urea (46% N) was applied twice at 75 kg·ha⁻¹, at sowing time and in the canola vegetative phase. Foliar applications of Prothioconazole® and Tebuconazole® (210 g·L⁻¹) against the fungal disease blackleg caused by *Leptosphaeria maculans* and Pirimicarb® (500 g·kg⁻¹) against aphids were applied as needed.

The experimental design was a complete randomised block with 26 genotypes (Sturt-TT was not included) and with three replicates in a spatially optimised layout of 5 ranges × 15 rows. Plot size was 1.6 m wide by 8 m long with 6 rows at a row spacing of 22 cm. On September 7, 2017 (100 days of sowing), canola and annual ryegrass densities were recorded in three random quadrats of 1 m by 1 m per plot. Canola biomass was determined from ten plants in the middle row of the plot whereas weed biomass was determined from three quadrats of 1 m by 1 m per plot. Both canola and weed biomass were cut at early canola flowering on September 20, 2017 and dried at 70°C for 48 h. Plant height of canola was recorded twice in each plot by measuring the height of 10 randomly-selected plants per plot at early flowering and at crop maturity stages. The time of 50% canola flowering of each genotype was recorded (mid-September to mid-October). Plots were harvested at maturity with a small-plot header (Kingaroy, Australia) and grain yield was expressed in g plot⁻¹.

3. Statistical Analysis

Glasshouse trial: Data were analysed using R software [32] and the ASReml package [33]. Graphics were produced in the lattice package [34]. The typical ASReml mixed model employed had fixed effects for genotype, weed (weedy v weed-free), and their interaction. Random effects were included (where significantly different from zero) for main plots and subplots.

Field trial: A Shapiro-Wilk test was used to examine the data for normality and scatter plot of residual versus fitted value was used to assess the homoscedasticity. On confirming data normality, the ASReml package in R was used to perform a linear mixed effect model. The observed data for crop shoot biomass (g plot⁻¹) and crop height (cm plant⁻¹) were normally distributed, whereas the data for yield (g plot⁻¹) was transformed to obtain normal distribution. The Box-Cox transformation using the parameter lambda equal to 0.25 was used to transform yield data. The aim of the analysis was to examine both the genotype effects and weed biomass while accounting for the block stratification and spatial layout of the plots (including ryegrass density across plots). The mean with 95% confidence interval for yield plot⁻¹, average crop height plot⁻¹ at flowering time and crop shoot biomass plot⁻¹ were presented graphically.

Correlation analysis: The Performance Analytics package in R was used for understanding their inter-relationships, including to describe their correlations in graphical format. The ggplot2 package in R was used to investigate the annual ryegrass response to 26 canola genotypes under the glasshouse and field conditions.

4. Results

4.1. Genetic Variation for Weedcompetitive Ability under Glasshouse Conditions

The analysis revealed highly significant differences between genotypes and between weed treatments for all traits evaluated, whereas the interaction between the genotype and weed treatments was only significant in stem biomass and plant height (Table 2). Several genotypes revealed a strong ability to interfere with ryegrass growth under glasshouse conditions. Significant genotypic effects were found on shoot biomass of ryegrass that ranged from 1.05 to 2.28 g plant⁻¹ (Figure 1(a)). Two winter varieties (cv. Akela and cv. Gross-Luesewitze) and a semi-winter variety (cv. Ningyou 7) were the most ryegrass suppressive genotypes, resulting in a lower annual ryegrass biomass of 1.05 to 1.26 g plant⁻¹, followed by three Australian spring varieties (Av-Garnet, Av-Opal and Tarcoola-141). However, the other three Australian spring varieties (Sturt-TT, Ag-Spectrum and Lantern) were least suppressive, having higher levels of ryegrass biomass (2.15 to 2.28 g plant⁻¹). These results suggested that growth habit of canola genotypes does not have any consistent relationship with ryegrass suppression.

Weed treatment influenced shoot biomass of canola genotypes as compared with the weed-free treatment (Figure 1(c)). However, the crop height was less affected by the presence of ryegrass (Figure 1(b)). This explains why crop biomass had higher negative correlation with weed biomass relative to the low correlation between crop height and ryegrass biomass. To identify competitive traits suitable for genetic selection, we sought correlation relationships between ryegrass and canola phenotypes. Leaf and shoot biomass showed a significant negative correlation ($r = -0.50$ to -0.76) with biomass of annual ryegrass (Figure 2). In contrast crop heights and leaf number had positive relationships with ryegrass biomass (Figure 2).

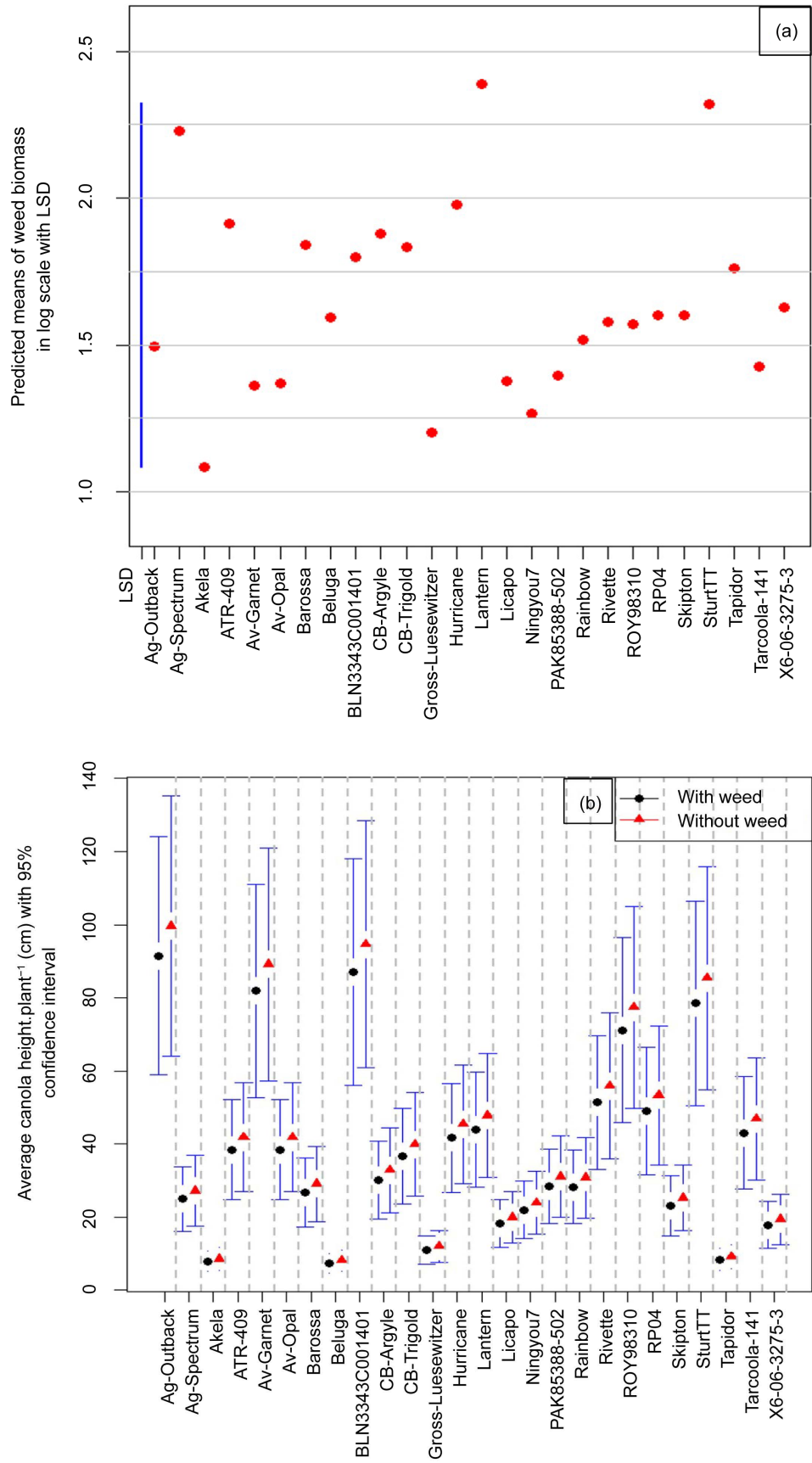
4.2. Validation of Genetic Variation for Weed Competitive Ability under Field Conditions

Our glasshouse experiment revealed that vigorous canola genotypes having

Table 2. *P*-values for fixed effects (genotype, weed treatment and genotype x weed interaction for each six traits measured under the glasshouse condition.

Effect	*Dry weed biomass (g plant ⁻¹)	Dry shoot biomass (g plant ⁻¹)	Dry leaf biomass (g plant ⁻¹)	Dry stem biomass (g plant ⁻¹)	Leaf (no. plant ⁻¹)	Plant height (cm plant ⁻¹)
Genotype	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Weed	n/a	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction	n/a	0.211	0.64	<0.001	0.624	0.001

*n/a, Not applicable; only weedy treatment are included in the analysis of weed biomass.



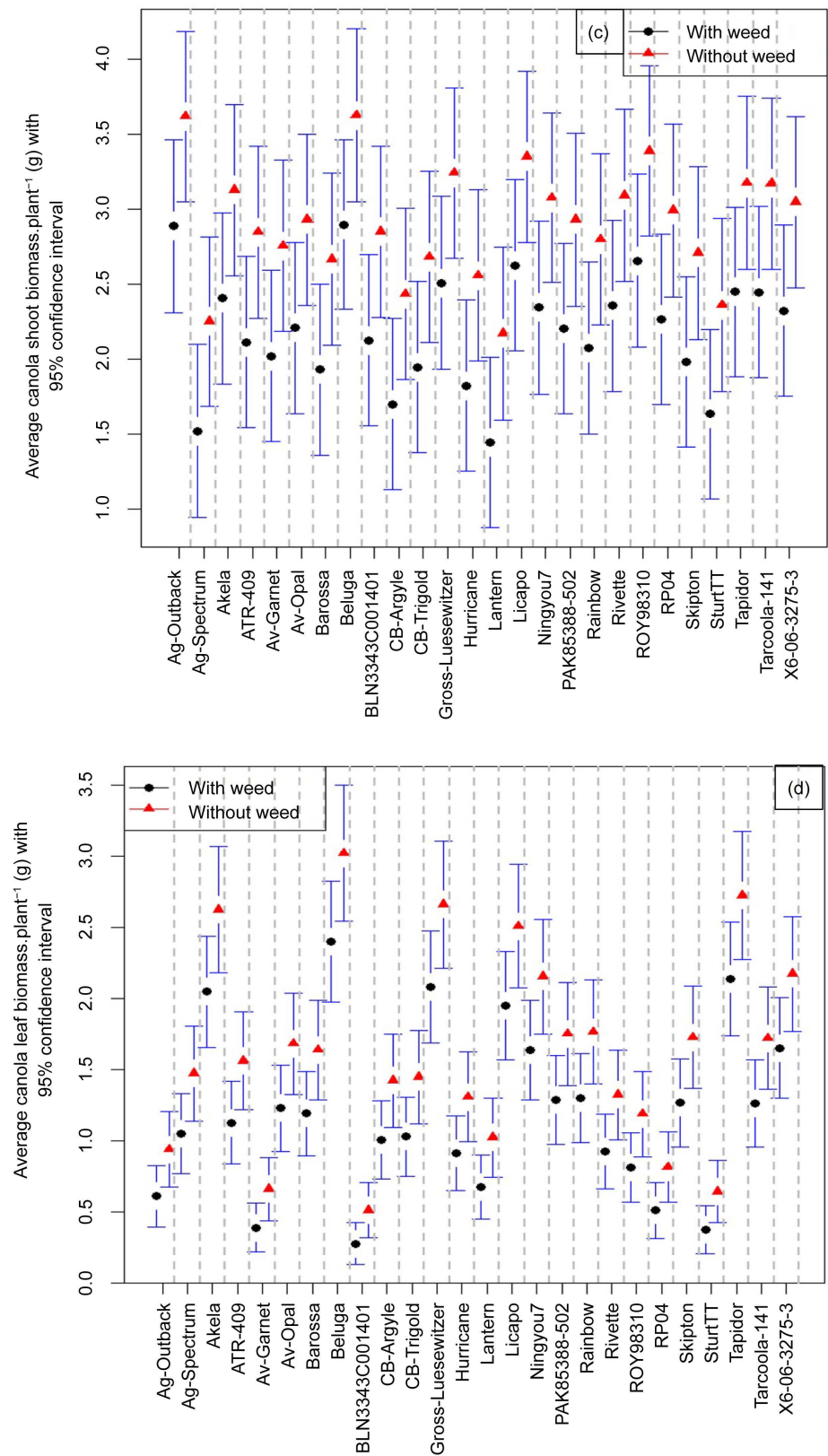


Figure 1. Weed competitiveness of the 26 canola genotypes against ryegrass. Predicted means from the fitted model described in **Table 1** are presented: (a) weed biomass (g plant⁻¹), (b) crop height (cm plant⁻¹), (c) crop biomass (g plant⁻¹) and (d) leaf biomass (g plant⁻¹), as predicted.

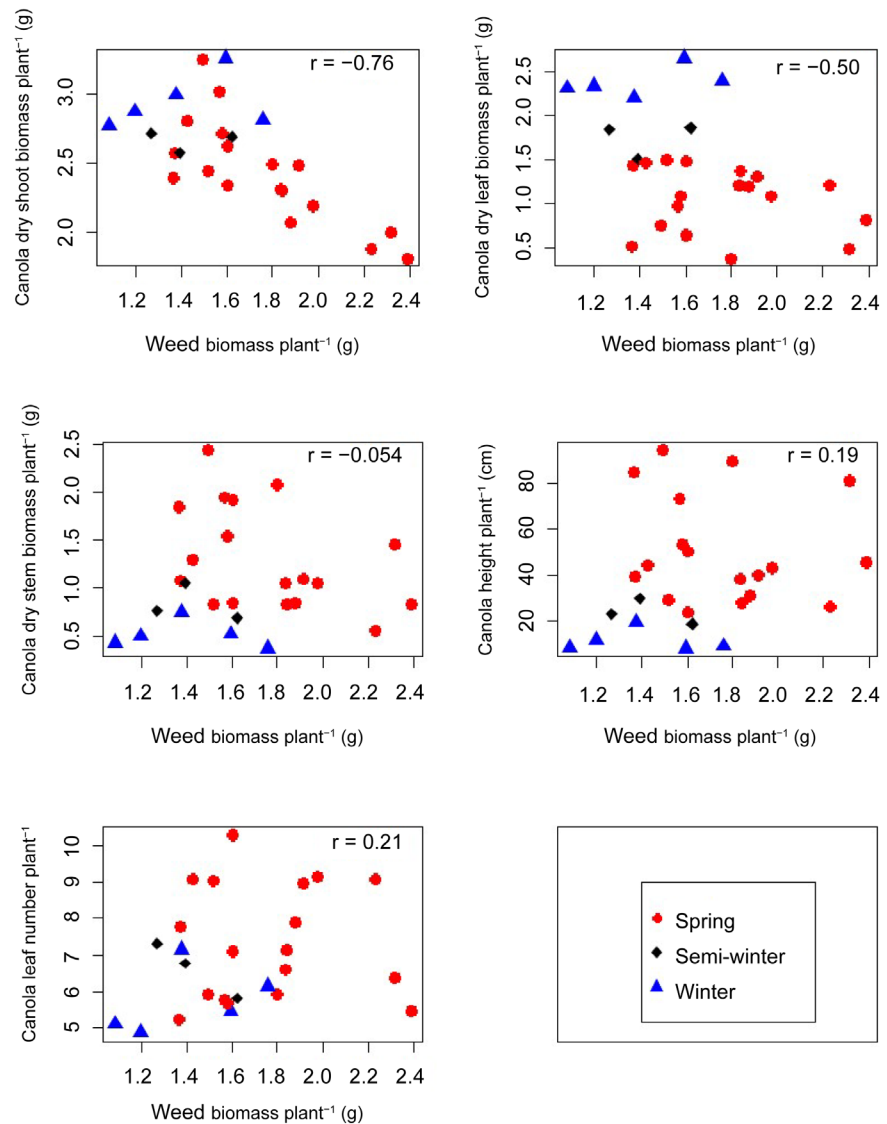


Figure 2. Correlation and correlation coefficient (r) of five traits (dry shoot biomass, dry leaf biomass, dry stem biomass, plant height and leaf number) for 26 canola genotypes and of biomass for annual ryegrass under the glasshouse condition.

higher leaf and shoot biomass control ryegrass better as compared to less vigorous genotypes. In order to validate the competitiveness of vigorous varieties against ryegrass, we conducted a field experiment using 26 canola genotypes. Results reconfirmed that plant vigour (shoot biomass) is a genetic trait and significantly affects ryegrass (shoot biomass), but the interaction between genotypes and weed biomass was non-significant (Table 3). This indicates that genotype and/or weed treatment is not influenced with growing environment (ryegrass influenced the crop biomass at a constant rate across all genotypes). As observed under glasshouse experiment, there were significant genotypic differences in plant height; however it did not significantly affect ryegrass (shoot biomass). The interaction between genotypes and weed biomass was also significant

Table 3. *P*-values for fixed effects (genotype) and covariate (weed biomass g plot⁻¹) and interaction between genotype and weed biomass (g plot⁻¹), crop biomass (g plot⁻¹), crop height (cm plant⁻¹) at flowering (growth stage 65, height 1) and physiological maturity (growth stage 80, height 2), measured under the field conditions.

Effect	Crop biomass (g plot ⁻¹)*	Crop height 1 (cm plant ⁻¹)*	Grain yield (g plot ⁻¹)*
Genotypes	<0.001	<0.001	<0.001
Weed biomass (g plot ⁻¹)	0.005	0.368	0.244
Interaction	0.132	<0.001	n/a
Crop biomass (g plot ⁻¹)	n/a	n/a	<0.001
Crop height 1 (cm plant ⁻¹)	n/a	n/a	0.02
Crop height 2 (cm plant ⁻¹)	n/a	n/a	<0.001
Flowering time	n/a	n/a	0.159

*n/a, Not applicable, covariate included in the analysis.

(Table 3). The main effect of genotypes on grain yield was highly significant, whereas the effects of weed biomass and flowering time were not significant. The significant genetic effects of crop biomass and plant height may have accounted the variation in yield (Table 3).

4.3. Correlation between Weed Biomass, and Canola Development and Productivity Traits under Field Conditions

Crop biomass and grain yield showed significantly negative correlations with ryegrass biomass. As anticipated, crop biomass was strongly correlated ($r = 0.55$) with grain yield (Figure 3). Four canola genotypes: Ag-Outback, Av-Opal, Skipton and X6-06-3275-3 showed the higher dry biomass, indicating that these genotypes have strong ability for ryegrass competitiveness. These genotypes produced higher grain yield as compared to cvs. Ag-Spectrum, Beluga, CB-Argyle, Lantern and Tapidor that had the lower biomass and grain yield (Figure 4(a) and Figure 4(c)). Early flowering resulted in the increase of yield and showed a negative correlation (Figure 3). Crop height measured at both flowering and physiological stages was positively correlated with yield, with correlation coefficient 0.74 and 0.67, respectively. Five canola genotypes, PAK85388-502, Rivette, Av-Garnet, Tarcoola-141 and X6-06-3275-3, were taller and those genotypes had the higher yields (Figure 4(b) and Figure 4(c)). However, ryegrass biomass was poorly correlated with crop height at the flowering and physiological maturity (Figure 3). Winter varieties were the shortest compared with semi-winter and spring varieties which could be attributed to their vernalisation requirement.

4.4. Correlation between Weed Suppression Ability of Canola Genotypes under the Glasshouse and Field Conditions

A positive relationship was observed between ryegrass and crop biomass data

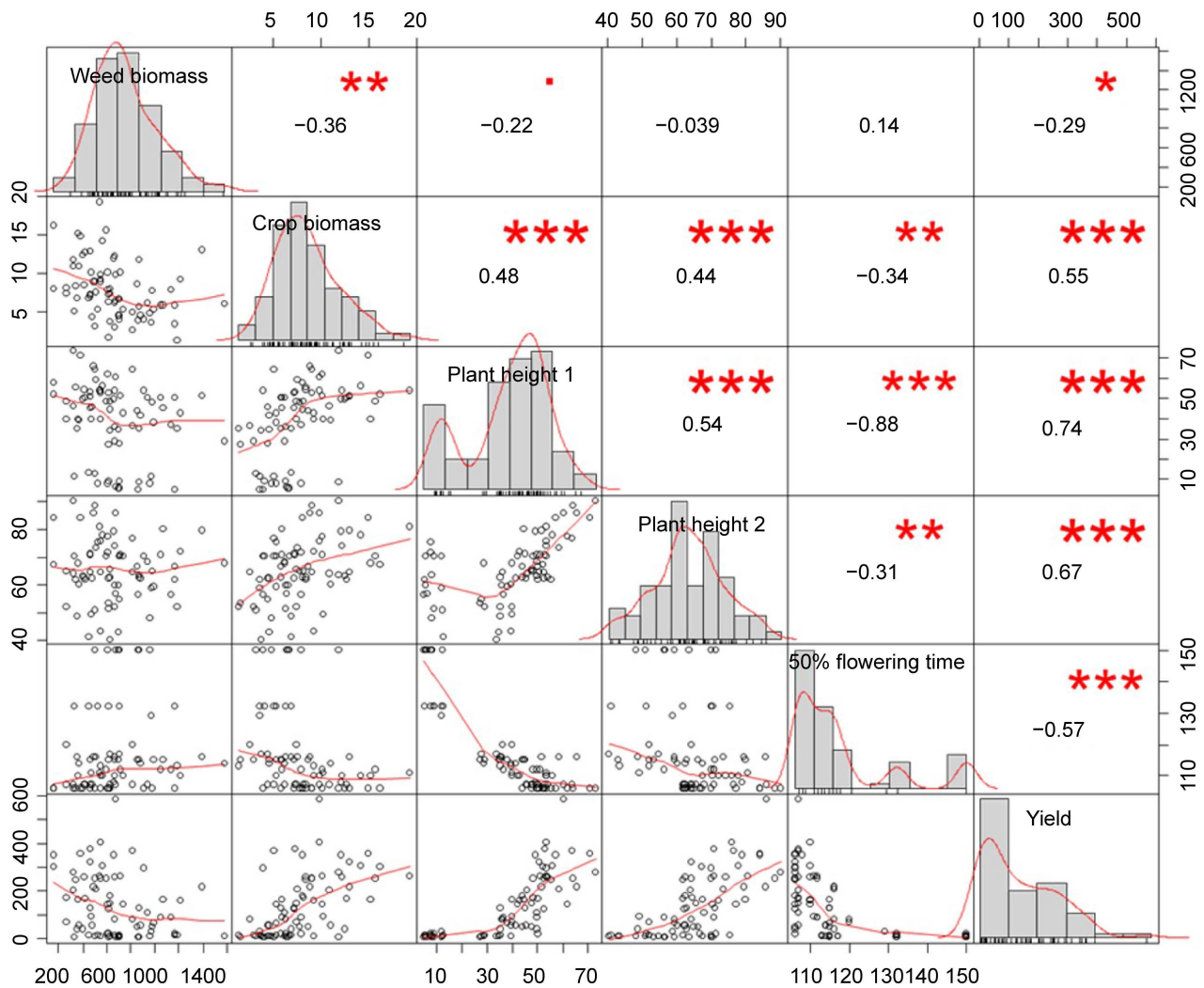
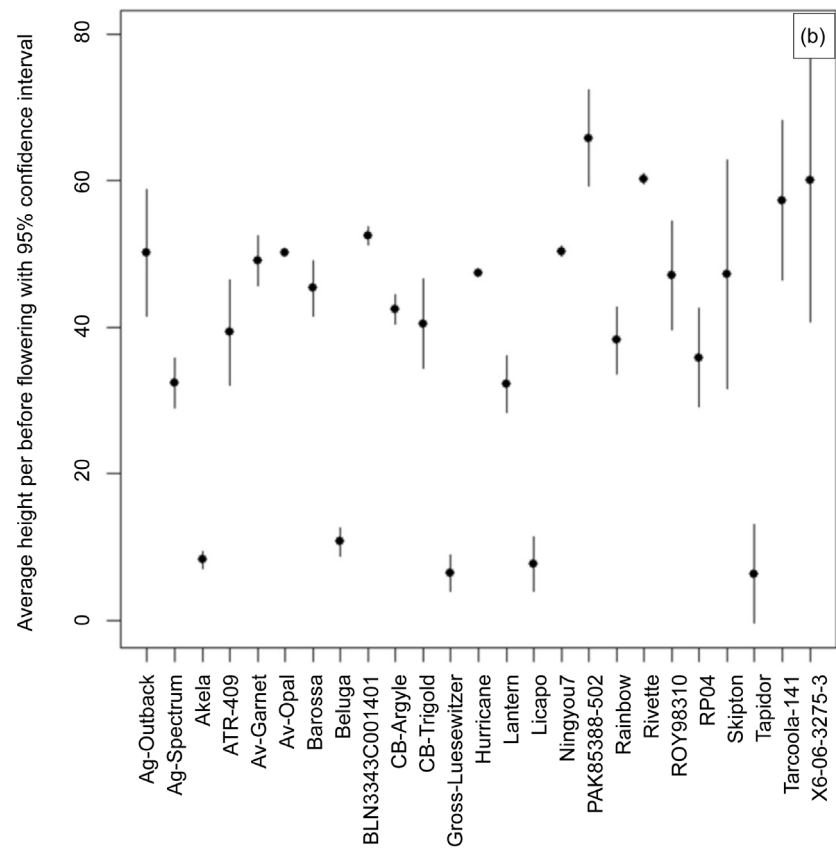
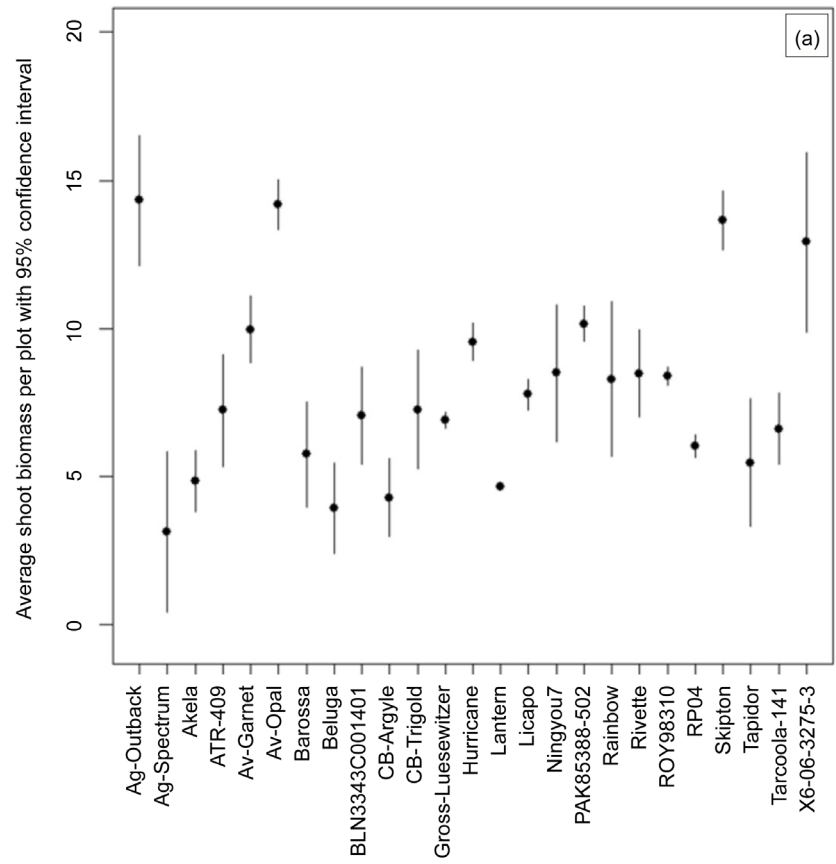


Figure 3. Correlation, correlation coefficient and normal distribution of five traits for 25 canola genotypes and of biomass for annual ryegrass grown under the field condition.

collected under glasshouse and field conditions (Figure 5). Our results showed that five canola genotypes: Akela, PAK85388-502, Av-Opal, Av-Garnet and Tarcoola-141 had lower weed biomass under both conditions, whereas Ag-Spectrum had the highest weed biomass. There was higher biomass of ryegrass in Lantern under the glasshouse condition but it was moderate under the field condition and this may have attributed to the variable density of ryegrass and require further verification.

5. Discussion

Weed biomass, crop height, crop biomass and yield are the common proxy traits used to screen canola genotypes for the weed-crop competition under field conditions. However, field-based screening has some limitations which influence the reliability and accuracy of phenotypic estimations. Screening different germplasm for weed-crop competition under glasshouse conditions can provide



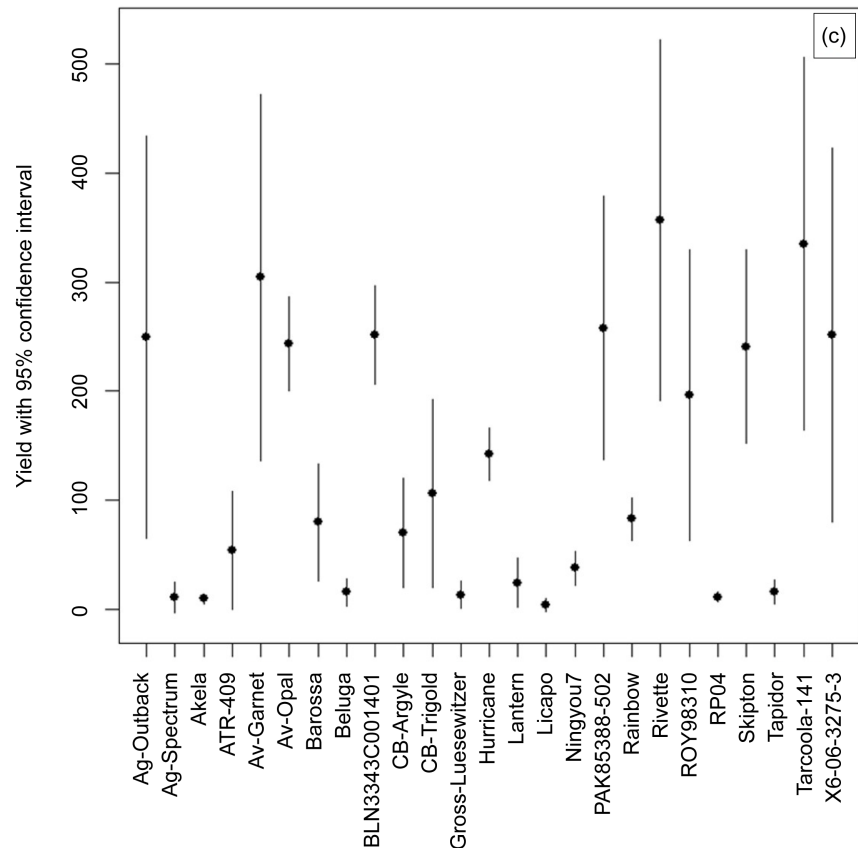


Figure 4. The 25 canola genotypes means with 95% confidence interval for: (a) crop biomass (g plot^{-1}) as predicted from the fitted model: canola genotypes * weed biomass (g plot^{-1}), (b) crop height at flowering stage (cm plant^{-1}) as predicted from the fitted model: canola genotype * weed biomass (g plot^{-1}), (c) grain yield (g plot^{-1}) as predicted from the fitted model: canola genotypes + weed biomass (g plot^{-1}) + crop biomass (g plot^{-1}) + crop height at physiological stage (cm plant^{-1}) + crop height at flowering stage (cm plant^{-1}) + time to 50% flowering.

some advantages over field conditions. In our study we compared the competitiveness of 26 *Brassica* genotypes to suppress ryegrass under glasshouse and field conditions. For most traits, genotypes and weed treatments had a high significant effect. The interaction was only significant on crop height in both conditions. Five genotypes: Akela, Tarcoola-141, Av-Opal, Av-Garnet and PAK85388-502 consistently showed the ability to suppress weed ryegrass in both glasshouse and field conditions. Majority of these varieties had also the highest grain yields in the field conditions except in Akela.

A good understanding of morphology and phenology of both weed and crop could lead to an improvement in weed-crop competition via the use of genetic and genomic tools. In cereals, extensive leaf display, leaf area index, long flag leaves and good ground cover have been associated with superior competitive ability [31] [35] [36]. Morphological traits associated with the interception of radiation by leaves including plant height, leaf size, number and leaf area index, have been implicated in competitiveness for light [26]. Our study showed a

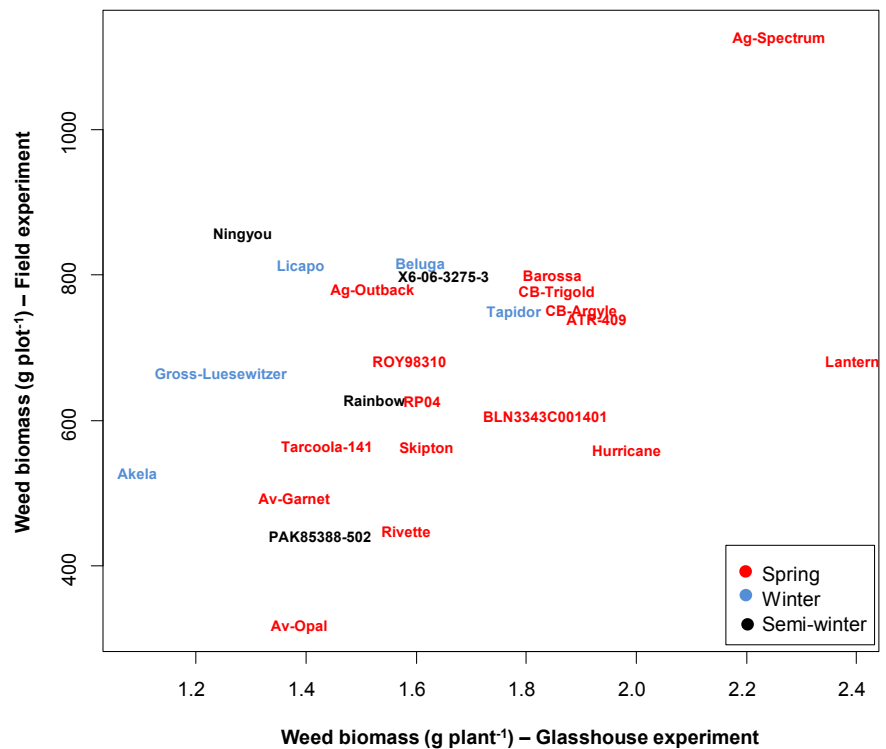


Figure 5. Correlation between two set of data of annual ryegrass biomass grown with 25 canola genotypes under the glasshouse and field conditions.

significant correlation between crop biomass and ryegrass suppression in both glasshouse and field conditions. These results indicate that plant vigour, manifested in higher canola biomass is associated with suppression of annual ryegrass. This is in agreement with previous studies where diverse canola accessions were compared under field conditions and showed that early-season crop biomass accumulation (early vigour) was associated with weed suppression [16] [17] [22]. In the glasshouse experiment, we found that leaf biomass was significantly correlated with weed suppression ($r = -0.50$), whereas crop stem biomass did not show any correlation ($r = -0.05$). Number of leaves also was not correlated with weed suppression. These results indicate that larger leaves are likely to provide shade and thus interfere with light inception to ryegrass. Further studies to understand the association between the area and angle of leaves and the suppression of weed growth are required for the germplasm used in our study. This would identify effective phenotypic traits for weed competition in a breeding program. Under field conditions, increase in crop biomass was associated with reductions in ryegrass biomass and with increased crop yields. This may reveal that canola biomass and particularly leaf biomass related to weed suppression and consequently improvement in yield.

The glasshouse and field experiments showed a low correlation between crop height and weed suppression but no correlation between crop stem biomass and weed suppression. Such lack of relationship has also been found in screening 111 rice genotypes against *Echinochloa crus-galli* in the field [37]. However, this re-

sult is not in agreement with previous studies in canola. The glasshouse experiment (**Figure 1(b)**) indicated that crop height was less affected by the presence of weed compared with those in weed free conditions, whereas crop biomass was highly affected by weed (**Figure 1(c)**). Similarly, in the field experiment, crop height was not significantly influenced by the weed, whereas the crop biomass was. Most winter varieties found in our study are good weed suppressors in the glasshouse experiment (**Figure 1(a)**) and only Akela was superior in weed suppression in the field experiment (**Figure 4**). A spring genotype, Tarcoola-141 showed better ability in weed suppression under the glasshouse and field conditions with higher yield in the field. Other two spring genotypes, Av-Opal and Av-Garnet and a semi-winter PAK85388-502 demonstrated good weed suppression under both conditions as it was found in other studies [9] [17]. Three spring genotypes including Ag-Spectrum, Lantern and Sturt-TT were found less to be weed suppressive under glasshouse and field conditions and had low grain yield in the presence of weeds.

6. Conclusion

Our study suggests that: 1) vigorous canola varieties can provide competitiveness to ryegrass under glasshouse and field conditions and 2) glasshouse conditions can be used to evaluate weed suppression ability of a large number of canola accessions while maintaining uniform density of annual ryegrass. We conclude that vigorous genotypes with highest weed suppression can be exploited for weed control in canola. Further research is required to develop structured populations between highest and lowest weed suppression genotypes can be developed to understand genetics underlying this valuable trait. Molecular markers can be developed for marker assisted selection leading to an acceleration of improved varietal development pipeline.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] AOF (2017) Australian Oilseeds Federation. Crop Report. http://www.australianoilseeds.com/_data/assets/pdf_file/0012/11190/AOF_Crop_Report_July_2017.pdf
- [2] Lemerle, D., Blackshaw, R., Smith, A., Potter, T. and Marcroft, S. (2001) Comparative Survey of Weeds Surviving in Triazine-Tolerance and Conventional Canola Crops in South-Eastern Australia. *Plant Protection Quarterly*, **16**, 37-40.
- [3] Llewellyn, R.R.D., Ouzman, J., Walker, S., Mayfield, A. and Clarke, M. (2016) Im-

Impact of Weeds on Australian Grain Production: The Cost of Weeds to Australian Grain Growers and the Adoption of Weed Management and Tillage Practices. Report to GRDC and CSIRO, Australia.

- [4] Broster, J., Pratley, J., Ip, R., Ang, L. and Seng, K. (2019) A Quarter of a Century of Monitoring Herbicide Resistance in *Lolium rigidum* in Australia. *Crop and Pasture Science*, **70**, 283-293. <https://doi.org/10.1071/CP18584>
- [5] Heap, I. (2017) The International Survey of Herbicide Resistant Weeds. <http://www.weedscience.org>
- [6] Donald, C. (1963) Competition among Crop and Pasture Plants. In: Norman, A.G., Ed., *Advances in Agronomy, Volume 15*, Academic Press, New York, 1-118. [https://doi.org/10.1016/S0065-2113\(08\)60397-1](https://doi.org/10.1016/S0065-2113(08)60397-1)
- [7] Pratley, J.E. (1996) Allelopathy in Annual Grasses. *Plant Protection Quarterly*, **11**, 213-214.
- [8] Asaduzzaman, M., An, M., Pratley, J.E., Luckett, D.J. and Lemerle, D. (2014) Canola (*Brassica napus*) Germplasm Shows Variable Allelopathic Effects against Annual Ryegrass (*Lolium rigidum*). *Plant Soil*, **380**, 47-56. <https://doi.org/10.1007/s11104-014-2054-4>
- [9] Asaduzzaman, M., Luckett, D., Cowley, R., An, M. and Pratley, J. (2014) Canola Cultivar Performance in Weed-Infested Field Plots Confirms Allelopathy Ranking from *in Vitro* Testing. *Biocontrol Science and Technology*, **24**, 1394-1411. <https://doi.org/10.1080/09583157.2014.942596>
- [10] Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. and Vivanco, J.M. (2006) The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annual Review of Plant Biology*, **57**, 233-266. <https://doi.org/10.1146/annurev.arplant.57.032905.105159>
- [11] Lemerle, D., Verbeek, B., Cousens, R.D. and Coombes, N.E. (1996) The Potential for Selecting Wheat Varieties Strongly Competitive against Weeds. *Weed Research*, **36**, 505-513. <https://doi.org/10.1111/j.1365-3180.1996.tb01679.x>
- [12] Asaduzzaman, M., Luckett, D.J., An, M., Pratley, J.E. and Lemerle, D. (2014) Management of Paterson's Curse (*Echium plantagineum*) through Canola Interference. In: *Nineteenth Australasian Weeds Conference*, Hobart, 162-165.
- [13] Seal, A.N. and Pratley, J.E. (2010) The Specificity of Allelopathy in Rice (*Oryza sativa*). *Weed Research*, **50**, 303. <https://doi.org/10.1111/j.1365-3180.2010.00783.x>
- [14] Wu, H., Pratley, J.E., Lemerle, D. and Haig, T. (2000) Laboratory Screening for Allelopathic Potential of Wheat (*Triticum aestivum*) Accessions against Annual Ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research*, **51**, 259-266. <https://doi.org/10.1071/AR98183>
- [15] Asaduzzaman, M., Pratley, J.E., Min, A., Luckett, D.J. and Lemerle, D. (2014) Canola Interference for Weed Control. *Science Reviews*, **2**, 63-74. <https://doi.org/10.1007/s40362-014-0022-2>
- [16] Daugovish, O., Thill, D. and Shafii, B. (2002) Competition between Wild Oat (*Avena fatua*) and Yellow Mustard (*Sinapis alba*) or Canola (*Brassica napus*). *Weed Science*, **50**, 587-594. [https://doi.org/10.1614/0043-1745\(2002\)050\[0587:CBWOF\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050[0587:CBWOF]2.0.CO;2)
- [17] Lemerle, D., Luckett, D., Lockley, P., Koetz, E. and Wu, H. (2014) Competitive Ability of Australian Canola (*Brassica napus*) Genotypes for Weed Management. *Crop and Pasture Science*, **65**, 1300-1310. <https://doi.org/10.1071/CP14125>
- [18] Raman, H., Shamaya, N. and Pratley, J. (2018) Genetic Variation for Weed Compe-

- tion and Allelopathy in Rapeseed (*Brassica napus* L.). In: Radhakrishnan, R., Ed., *Biological Approaches for Controlling Weeds*, IntechOpen, London, United Kingdom. <https://doi.org/10.5772/intechopen.79599>
- [19] Lutman, P.J.W., Dixon, F.L. and Risiott, R. (1994) The Response of Four Spring-Sown Combination Arable Crops to Weed Competition. *Weed Research*, **34**, 137-146. <https://doi.org/10.1111/j.1365-3180.1994.tb01981.x>
- [20] Melander, B. (1993) Modelling the Effects of *Elymus repens* (L.) Gould Competition on Yield of Cereal, Peas and Oilseed Rape. *Weed Research*, **33**, 99-108. <https://doi.org/10.1111/j.1365-3180.1994.tb01977.x>
- [21] Lemerle, D.V. and Coombes, N.E. (1995) Losses in Grain Yield of Winter Crops from *Lolium rigidum* (Gaud.) Depend on Crop Species, Cultivar and Season. *Weed Research*, **35**, 503-509. <https://doi.org/10.1111/j.1365-3180.1995.tb01648.x>
- [22] Beckie, H.J., Johnson, E.N., Blackshaw, R.E. and Gan, Y. (2008) Weed Suppression by Canola and Mustard Cultivars. *Weed Technology*, **22**, 182-185. <https://doi.org/10.1614/WT-07-126.1>
- [23] Harker, N.C., Blackshaw, R. and O'Donovan, J. (2003) Seeding Rate, Herbicide Timing and Competitive Hybrids Contribute to Integrated Weed Management in Canola (*Brassica napus*). *Canadian Journal of Plant Science*, **83**, 433-440. <https://doi.org/10.4141/P02-073>
- [24] Harker, N., O'Donovan, J., Blackshaw, R., Johnson, E., Holm, F. and Clayton, G. (2011) Environmental Effects on the Relative Competitive Ability of Canola and Small-Grain Cereals in a Direct-Seeded System. *Weed Science*, **59**, 404-415. <https://doi.org/10.1614/WS-D-10-00121.1>
- [25] Lemerle, D., Lockley, P., Koetz, E. and Wu, H. (2014) Competitive Ability of Australian Canola (*Brassica napus*) Genotypes for Weed Management. *Crop and Pasture Science*, **65**, 1300-1310. <https://doi.org/10.1071/CP14125>
- [26] Coleman, R., Gill, G. and Rebetzke, G.J. (2001) Identification of Quantitative Trait Loci for Traits Conferring Weed Competitiveness in Wheat (*Triticum aestivum* L.). *Australian Journal of Agricultural and Research*, **52**, 1235-1246. <https://doi.org/10.1071/AR01055>
- [27] Asaduzzaman, M., An, M., Pratley, J.E., Luckett, D.J. and Lemerle, D. (2014) Laboratory Bioassay for Canola (*Brassica napus*) Allelopathy. *Journal of Crop Science and Biotechnology*, **17**, 267-272. <https://doi.org/10.1007/s12892-014-0087-0>
- [28] Zand, E. and Beckie, J. (2002) Competitive Ability of Hybrid and Open-Pollinated Canola (*Brassica napus*) with Wild Oat (*Avena fatua*). *Canadian Journal of Plant Science*, **82**, 473-480. <https://doi.org/10.4141/P01-149>
- [29] Worthington, M. and Reberg-Horton, C. (2013) Breeding Cereal Crops for Enhanced Weed Suppression: Optimizing Allelopathy and Competitive Ability. *Journal of Chemical Ecology*, **39**, 213-231. <https://doi.org/10.1007/s10886-013-0247-6>
- [30] Gealy, D.R. and Yan, W. (2012) Weed Suppression Potential of 'Rondo' and Other Indica Rice Germplasm Lines. *Weed Technology*, **26**, 517-524. <https://doi.org/10.1614/WT-D-11-00141.1>
- [31] Johnson, D.E., Dingkuhn, M., Jone, M.P. and Mahamane, M.C. (1998) The Influence of Rice Plant Type on the Effect of Weed Competition on *Oryza sativa* and *Oryza glaberrima*. *Weed Research*, **38**, 207-216. <https://doi.org/10.1046/j.1365-3180.1998.00092.x>
- [32] Team, R.D.C. (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- [33] Butler, D.G., Cullis, B.R., Gilmour, A.R. and Gogel, B.J. (2009) ASReml-R Reference

- Manual. Release 3.0. Technical Report, Queensland Department of Primary Industries, Australia. <http://www.vsni.co.uk/downloads/asreml/release2/doc/asreml-R.pdf>
- [34] Sarkar, D. (2008) Lattice: Multivariate Data Visualization with R. Springer, New York. <https://doi.org/10.1007/978-0-387-75969-2>
- [35] Donald, C.M. and Hamblin, J. (1976) Biological Yield and Harvest Index of Cereal as Agronomic and Plant Breeding Criteria. *Advances in Agronomy*, **28**, 361-405. [https://doi.org/10.1016/S0065-2113\(08\)60559-3](https://doi.org/10.1016/S0065-2113(08)60559-3)
- [36] Huel, D.G. and Hucl, P. (1996) Genotypic Variation for Competitive Ability in Spring Wheat. *Plant Breeding*, **115**, 325-329. <https://doi.org/10.1111/j.1439-0523.1996.tb00927.x>
- [37] Olofsson, M., Navarez, D., Rebulanan, M. and Streibig, J. (1999) Weed-Suppressing Rice Cultivars: Does Allelopathy Play a Role? *Weed Research*, **39**, 441-454. <https://doi.org/10.1046/j.1365-3180.1999.00159.x>

Phenotypic, Cytological and Molecular (AFLP) Analyses of the Cotton Synthetic Allohexaploid Hybrid (*G. hirsutum* × *G. longicalyx*)²

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Abstract

The wild cotton diploid species ($2n = 2x = 26$) are important sources of useful traits such as high fiber quality, resistance to biotic and abiotic stresses etc., which can be introgressed into the cultivated tetraploid cotton *Gossypium hirsutum* L ($2n = 4x = 52$), for its genetic improvement. The African wild diploid species *G. longicalyx* Hutchinson and Lee could be used as donor of the desirable traits of fiber fineness and resistance to reniform nematode. However, hybridization of wild diploid species and cultivated tetraploid cotton encounters a sterility problem of the triploid ($2n = 3x = 59$), mainly due to ploidy. The restoration of the fertility can be done by creating an allohexaploid ($2n = 6x = 78$) through the doubling with colchicine of the sterile triploid chromosomes. With this method, a synthetic allohexaploid hybrid (*G. hirsutum* × *G. longicalyx*)² has been obtained. This genotype was studied using phenotypic, cytological and molecular (AFLP) analyses in order to confirm its hybridity and its karyotype, and also to verify the expression of the desirable traits coming from *G. longicalyx*. The studied genotype showed a quite good level of pollen fertility (83%), and apart from larger seeds and some minor seedling anomalies, most of its morphological characteristics were intermediate between the two parental species. It had 78 chromosomes, proving its hexaploid status. Molecular analysis revealed 136 AFLP loci in this hexaploid, all from *G. hirsutum* and *G. longicalyx*, demonstrating its hybrid status. In addition, the hexaploid exhibited the useful traits of *G. longicalyx* with regard to its remarkable fiber fineness and its high resistance to the reniform nematode. This allohexaploid hybrid constitutes an interesting agronomic material, which can be used as a bridge for the transfer of useful agro-

onomic traits from wild species to varieties of *G. hirsutum*.

Keywords

Gossypium spp, Hexaploid Hybrid, Chromosomes, AFLP Marker, Plant Breeding

1. Introduction

Cotton, from the genus *Gossypium*, is the most important natural fiber source for the textile industry in the world [1]. The *Gossypium* genus is composed of 53 species among which 7 are tetraploid ($2n = 4x = 52$) and 46 are diploid ($2n = 2x = 26$) [2]. Diploid cottons are classified into 8 genome types, denoted A-G and K, based on chromosome pairing relationships [3] [4]. The tetraploid cottons have a genome designated by AD, which resulted from the ancestral allopolyploidization of progenitor A-genome and D-genome diploids about 1-2 million years ago [2] [5].

Two cotton diploids species (*G. arboreum* L and *G. herbaceum* L) and two tetraploids species (*G. hirsutum* L and *G. barbadense* L) are cultivated for their spinnable fiber [6] [7] [8]. *G. hirsutum*, known as Upland cotton, provides more than 90% of the world's cotton production due to its high yield [8]. The remaining cotton fiber supply is produced from the other three cultivated cottons [9]. Apart from these four species, the other 46 species of *Gossypium* are wild.

Genetic improvement of the main cultivated cotton *G. hirsutum* can be done using wild species as donor of traits of interest [10]. Indeed, wild diploid species are important sources of several desirable genes to improve fiber quality, resistance to diseases and insect pests, or tolerance to abiotic stress of Upland cotton [4] [11]. The African wild species *G. longicalyx* Hutchinson and Lee (F1 genome) represents an interesting source of genes that can potentially be transferred to the main cultivated cotton species. This wild diploid species could be used as donor of the desirable trait of fiber fineness, which is very important to textile industry [4] [12] [13], and also as donor of the resistance to reniform nematode [14] [15].

Technically, the use of wild diploid species to improve cultivated tetraploid cotton faces the problem of the sterility of the triploid ($2n = 3x = 59$) obtained, mainly due to ploidy. To overcome this problem, a strategy used involves the creation of an allohexaploid hybrid as a bridge genotype. This method begins with the hybridization of *G. hirsutum* with a diploid wild species to obtain a sterile triploid ($2n = 3x = 59$). The next step consists in doubling the chromosome number of the sterile triploid with colchicine to give a fertile allohexaploid ($2n = 6x = 78$). Such a synthetic allohexaploid hybrid could subsequently be used to introgress the alien genes through trispecific hybrid or monosomic addition lines [16] [17] [18]. In cotton breeding, the successful use of this method has

been demonstrated by the effective introgression of alien chromosomal material into upland cotton and the expression of the beneficial effects of exotic genes [4] [11] [19] [20].

At the Laboratory of Tropical Agro ecology of Gembloux Agro-Bio Tech (Liège University, Belgium) a synthetic allohexaploid hybrid (*G. hirsutum* × *G. longicalyx*)² has been created. As a prelude to the use of this hexaploid in a breeding program, the present study aimed at its phenotypic, cytological and molecular analysis (AFLP markers) in order to confirm its hybridity and check its expression of the desirable traits from *G. longicalyx*.

2. Material and Methods

2.1. Plant Material

Plant material included plants of a synthetic allohexaploid hybrid (*G. hirsutum* × *G. longicalyx*)² and its parental species *G. longicalyx* and *G. hirsutum*. These plants were obtained from seeds coming from the collection of the Tropical Agro Ecology Laboratory of Gembloux Agro-Bio Tech (University of Liège, Belgium). In the crossing scheme used to generate the allohexaploid hybrid (*G. hirsutum* × *G. longicalyx*)², the tetraploid *G. hirsutum* (A₁A₁D₁D₁, 2n = 4× = 52) was crossed with the diploid *G. longicalyx* (F₁F₁, 2n = 2× = 26) to give triploid hybrid (A₁D₁F₁, 2n = 3x = 39) seeds. Hybrid seedling plants were then treated with 0.15% colchicine for chromosome doubling and an allohexaploid (A₁A₁D₁D₁F₁F₁) with 78 chromosomes was obtained. This putative synthetic allohexaploid hybrid produced flowers and set bolls normally. Its seeds were used to produce the plants that are investigated in the present study. The plants were grown in greenhouse, in 5 liter pots filled with a 3:2:1 (v:v:v) sterile mixture of compost, sand and peat.

2.2. Cytological Analysis

To check the chromosome numbers of the hexaploid and its parental species, mitotic chromosome preparations were carried out using root tips. Fast-growing root tips were collected in 0.04% 8-hydroxyquinoline for 4 hours at room temperature (RT) and fixed for 48 h in a fresh fixative fluid (3:1 ethanol:acetic acid) at 4°C. After washing in distilled water (2 × 10 min), treating in 0.25 N HCl (10 min), rinsing in distilled water (10 min) and treating in a 0.01M citrate buffer (10 min), root tips were digested in an enzyme solution (5% cellulase Onozuka R-10, 1% pectolyase Y-23 in citrate buffer) at 37°C for 1 hour. The enzyme mix was removed by rinsing in distilled water for 10 min, and on a clean glass slide a single root tip was spread in one or two drops of fresh fixative (3:1 ethanol:acetic acid) using a fine-pointed forceps. After staining with 4,6-diamino-2-phenylindole/Vectashield, mitotic metaphase plates were visualized and the chromosomes were counted under fluorescent light with a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a JVC KY-F 58E camera (JVC, Yokohama, Japan).

2.3. Phenotypic Observations

Morphological observation and plant fertility evaluation

The appearance, shape and size of the hexaploid seeds were observed and compared to those of its parental species and their germination rates were assessed. The test of germination was conducted on 44 seeds of the hexaploid and 30 seeds of each of its parental species. The seeds were placed to germinate in Petri dishes with moist filter paper at 28°C. The criterion for germination was a radicle length of > 1 mm. The germination rates were calculated as the percentage of seeds that germinated from the total number of seeds placed in the Petri dish.

The morphological observations carried out on the plants concerned: the aspect of seedlings (presence of malformations or not), the shape and the size of the leaves, the number of main stem nodes, the final plant height, the flower aspect and the capsule size.

For pollen fertility evaluation, about 300 pollen grains per plant were analyzed. Pollen grains collected in the morning on the day of anthesis were dipped in a drop of 1.5% acetic-carmin solution on a slide for 30 minutes and were analyzed under a stereomicroscope Nikon Eclipse E800 (Nikon, Tokyo, Japan). Only fully stained and large pollen grains were scored as viable and non-aborted. The quantity of viable pollen was estimated as the percentage of stained pollen.

The self-fertility was assessed by determining the average number of seeds obtained per self-pollinated flower. The cross-fertility was assessed by counting the average number of seeds obtained per cross-pollinated flowers.

Fiber fineness analysis

For fiber fineness analysis, cotton fibers were harvested at full maturity and used for the analysis. The fibers were combed and a tuft of parallel fibers was cut from the seed. Their free points were also cut and the median region was placed on a slide and covered with a cover glass. We let one or two drops of 18% NaOH solution penetrate by capillarity into the fibers. The NaOH solution swells the fibers. The diameter of at least 100 fibers was then measured with the software NIS-Elements BR 2.30 (Nikon, Japan) using the Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a digital JVC KY-F 58E camera (JVC, Yokohama, Japan). The ribbon width was determined by dividing the mean of the diameters measured by the 1.3 Summers coefficient [4] [13] [21]. The data collected were subjected to the analysis of variance (ANOVA) using the software Statistica 7.1 (Stat Soft, France). The least significant difference (LSD) was used to establish differences between means at $P = 0.05$.

Evaluation of resistance to reniform nematode

The resistance to reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) was assessed following the protocol of [15]. Briefly, 30-days seedlings planted in 20-cm-diameter plastic pots, were inoculated with 6000 eggs of *R. reniformis*. Sixty days after inoculation, the soil was gently removed, the roots carrying the nematode eggsacs were weighed and the reniform nematode eggs were then extracted and

counted according to a NaOCl-Blender-Sieving-Centrifugation-Flotation method. For each plant, the number of eggs per gram root was determined. These numbers were used to assess the relative resistance of each plant compared to the susceptible control *G. hirsutum* by calculating the percentage of eggs per gram root for each plant considering the 100% level for the susceptible control. The scale of relative resistance used contains the following classes: 0 - 10% = highly resistant, 11 - 25% = resistant, 26 - 40% = moderately resistant, 41 - 60% = low susceptible, 61 - 100% = susceptible as control, and above 100% = very susceptible. As this technique for evaluating resistance to reniform nematode was destructive, before their evaluation the plants were grafted onto vigorous seedlings of *G. hirsutum* in order to keep a copy of each of them.

2.4. Molecular Analysis

DNA isolation

Total genomic DNA of *G. hirsutum*, *G. longicalyx* and two synthetic allohexaploid (*G. hirsutum* × *G. longicalyx*)² plants were isolated from young fresh leaf tissues following the CTAB method as described by [22].

AFLP Analysis

AFLP was performed by Automated Laser Fluorescence (ALF) analysis. Three AFLP primer combinations were used: E-ACC/M-CAG, E-ACT/M-CTG and E-ACT/M-CAG. AFLP was carried out using the “AFLP Analysis System I / AFLP starter primer kit” (Invitrogen, Belgium) following the protocol proposed by Invitrogen. Briefly, genomic DNA (250 ng) was double digested with EcoR I and Mse I restriction endonucleases. The digested DNA fragments were ligated to EcoR I and Mse I adaptors with T4 DNA ligase to generate template DNA for amplification by PCR. Two consecutive PCR were performed: a pre-selective and selective PCR. In the pre-selective reaction, DNA was amplified using an AFLP pre-amp primer pair complementary to the adaptors and each having one selective nucleotide. Pre-selective PCR amplification was used as template for the selective amplification using AFLP primers, each containing three selective nucleotides. The PCR amplification products were run on 6% denaturing polyacrylamide gel using the ALF-Express (Pharmacia Biotech, Freiburg, Germany), which is an Automated Laser Fluorescence DNA sequencer. The obtained digital image of the profiles was analyzed. The scoring of bands was done as present (1) or absent (0) for AFLP marker loci and data were entered in a binary data matrix as discrete variables.

3. Results and Discussion

3.1. Mitotic Chromosome Analysis

Analysis of the mitotic metaphase plates showed 52 chromosomes for *G. hirsutum*, 26 chromosomes for *G. longicalyx* and 78 chromosomes for the putative (*G. hirsutum* × *G. longicalyx*)² hexaploid hybrid (Figure 1). This number of 78 chromosomes proves the hexaploid status of the material studied because it is in

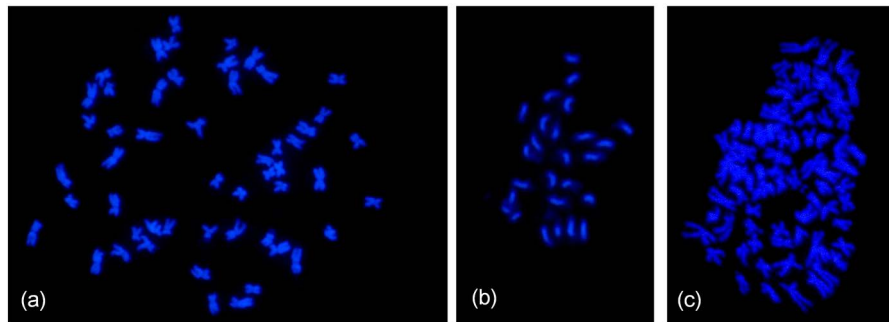


Figure 1. Chromosomal configuration at mitotic metaphase: (a) 52 chromosomes of *G. hirsutum* ($\times 1500$); (b) 26 chromosomes of *G. longicalyx* ($\times 1000$); (c) 78 chromosomes of the $(G. hirsutum \times G. longicalyx)^2$ hexaploid hybrid ($\times 1500$).

agreement with the number of chromosomes expected for a synthetic hexaploid cotton ($2n = 6x = 78$) resulting from the doubling of the chromosomes of a triploid issued from the cross between a tetraploid cotton (*G. hirsutum*, $2n = 4x = 52$) and a diploid cotton (*G. longicalyx*, $2n = 2x = 26$). This result confirms that artificial somatic allopolyploidization can be successfully accomplished *in vivo* through the use of antimitotic reagents, *i.e.* metaphase inhibitors such as colchicine [23] [24]. This reagent causes the depolymerization of the microtubular cytoskeleton in the early phases of metaphase, blocking the separation of chromosomes in mitoses, consequently leading to polyploidization of the cells [25].

3.2. Seed Aspect, Germination Rate and Seedling Abnormalities Analysis

The seeds of the $(G. hirsutum \times G. longicalyx)^2$ hexaploid hybrid had normal appearance and shape but they were all larger than the seeds of the parental species (Figure 2). This result is in accordance with [24] and [26] who reported that polyploids have usually larger seeds than parental species. It is probably a direct consequence of large cell size in polyploids [27], since genome duplication increases cell volume by increasing genome size [28]. This is in line with the expectation that the sizes of seed are a function of cell size, which is larger in polyploids [29].

In the germination test, the seeds of the hexaploid hybrid showed the relative lowest germination rate with 38 germinated seeds on 44 (86.36%) compared to its parental species *G. hirsutum* and *G. longicalyx*, which respectively presented 100% and 96.67% (29 germinated seeds on 30) germination rates. The germination rate gives an estimate of the viability of the seeds. The result obtained suggests a problem of viability of about 13% of the seeds produced by the allohexaploid hybrid. [24] working on synthetic polyploidy in *Hylocereus monacanthus* also reported problems of seed viability. The genome of newly formed polyploid plants usually undergoes extensive genetic and epigenetic changes which can alter gene expression and generate physiological changes that can affect seed viability [28] [30] [31].



Figure 2. Seeds of the hexaploid hybrid and its parental species: (a) smaller seed of *G. longicalyx*; (b) intermediate seeds size of *G. hirsutum*; (c) larger seeds of the hexaploid (*G. hirsutum* × *G. longicalyx*)².

Some of the hexaploid seedlings obtained from the 38 germinated seeds, presented some abnormalities at cotyledon leaves stage (Figure 3). Three types of abnormalities were observed: i) cotyledonary leaves welded on the petioles (Figure 3(a), 4 seedlings), ii) cotyledonary leaves welded on the entire length of the leaves (petioles and blades) giving the impression of a unique leaf (Figure 3(b) and Figure 3(c), 3 seedlings), iii) and seedlings with a normal cotyledonary leaf associated with a progressive necrosis of the other cotyledonary leaf (Figure 3(d), 3 seedlings). This necrosis consisted in a prolonged chlorosis of the cotyledonary leaf. It led to the premature death of the concerned leaves but not of the plant. However, [32] reported such an anomaly in polyploid wheat, where it ultimately caused the death of the whole plant in certain wheat hybrids. All abnormalities observed in the hexaploid seedlings were not observed in the parental *G. hirsutum* and *G. longicalyx* species. Out of 38 germinated hexaploid seeds, 10 seedlings showed one or other of the anomalies observed, *i.e.* 26.31% malformed seedlings. According to [33], such abnormalities could result in lethal or semi-lethal condition that imposes a great barrier when trying to transfer desirable traits between species. Actually, after polyploidization the architecture of the cell is modified, the cell must adapt to the new nuclear DNA content and deal with changes in the homology of the chromosomes, gene expression and epigenetics [25]. Changes in gene expression and physiological processes due to genome duplications can generate anomalies that can be deleterious for the development of the polyploid individual [25]. Fortunately, only 26.31% of the hexaploid plants in the present study presented anomalies, which were not necessarily fatal for the plants. Indeed, the malformations observed seemed to be minor and they had no detrimental consequences on the development of the plants except for a seedling with the abnormality of the fully welded cotyledonary leaves. This seedling remained at cotyledon leaves stage for a long time until death.

3.3. Analysis of Morphological Observations of the Plants

The height of the hexaploid plants varied from 152 to 236 cm with an average of

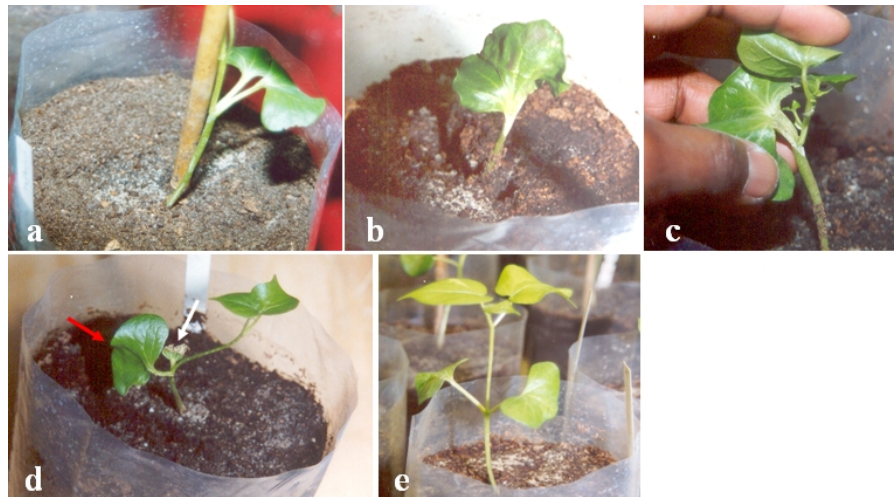


Figure 3. Pictures showing abnormalities observed in some seedlings of the hexaploid at cotyledon leaves stage: (a) a hexaploid seedling showing cotyledonary leaves welded at the level of the petioles; (b), (c) a hexaploid seedling having cotyledonary leaves welded on their entire length (petiole + blade); (d) a hexaploid seedling with a normal cotyledonary leaf (red arrow) and a cotyledonary leaf showing progressive necrosis (white arrow); (e) a hexaploid seedling with normal cotyledonary leaves.

192 cm, while the height of *G. hirsutum* and *G. longicalyx* were on average 82 cm and superior to 300 cm respectively. The hexaploid had 12 to 34 nodes (25 on average) on the main stem. The numbers of nodes on the main stem were on average 11 and 33 for *G. hirsutum* and *G. longicalyx* respectively. The hexaploid plants mostly carried tri-lobated leaves. The size of these leaves varied from 7.4 cm to 14.9 cm with an average of 10.54 cm. *G. hirsutum* presents the largest leaves (23.5 cm on average) with 3 - 5 lobes, while *G. longicalyx* had the smallest leaf (6 cm on average) with no lobe (simple leaves). The flowers of the hexaploid plants were a bit smaller than those of *G. hirsutum* but their general aspect was similar. *G. longicalyx* had the smallest flowers. The hexaploid produced relative smaller capsule than *G. hirsutum* but bigger than *G. longicalyx*. These results showed that most of the morphological characteristics exhibited by the synthetic allohexaploid hybrid were intermediate between *G. hirsutum* and *G. longicalyx*. This hexaploid hybrid did not generate transgressive morphological characters (characters which show values beyond the range of parental species). [20] studying the synthetic allohexaploid hybrid (*G. hirsutum* x *G. anomalum*)² also found that most of morphological characteristics of the hexaploid plants were intermediate between the two parental species. The intermediate morphology of hybrids compared to their parental species is a characteristic observed in several hybrids, since these characters are generally under polygenic control with simple additive effects [34] [35].

3.4. Fertility Analysis

The mean proportion of stainable pollen grains (pollen fertility) of the hexaploid plants was 83% while the pollen fertility of *G. hirsutum* and *G. longicalyx* ap-

proached 100%. The pollen fertility level of the hexaploid is quite good, even if it is relatively lower than that of the parental species. By selfing, the hexaploid gave a mean of 4.39 seeds per pollinated flowers while *G. hirsutum* and *G. longicalyx* produced respectively 34 and 6 seeds per capsule on average (Table 1). These results show the restoration of fertility at the hexaploid level by the doubling of the chromosomes of the sterile triploid hybrid, even if the self-fertility of the parental species was higher. The results of cross-pollinations between the hexaploid and *G. hirsutum* (Table 1) gave practically no seed per capsule, i.e. 0.018 and 0.3 seed per capsule when the hexaploid was used as female and male respectively. This very low success rate of cross-pollination, despite the good level of pollen fertilities (for both hexaploid hybrid and *G. hirsutum*) is probably due to the presence of incompatibility barriers between the hexaploid hybrid and *G. hirsutum* [36] [37].

3.5. Expression of Fiber Fineness and Resistance to Reniform Nematode Traits

The results of the fiber fineness analysis are presented in Table 2. The hexaploid (*G. hirsutum* × *G. longicalyx*)² showed a mean value of ribbon width of 12.53 μm. *G. longicalyx* had the finest fibers with 5.94 μm of ribbon width against 17.765 μm for *G. hirsutum*. These results show the interesting fiber fineness of

Table 1. Selfing of the hexaploid (*G. hirsutum* × *G. longicalyx*)² and its parental species, and backcrossing of the hexaploid to *G. hirsutum*.

Type of crossing	No of pollinated flowers	No of seeds harvested	No of seeds per pollinated flowers
Selfing of <i>G. hirsutum</i>	30	1020	34
Selfing of <i>G. longicalyx</i>	30	180	6
Selfing of (<i>G. hirsutum</i> × <i>G. longicalyx</i>) ²	23	101	4.39
(<i>G. hirsutum</i> × <i>G. longicalyx</i>) ² ♀ × <i>G. hirsutum</i> ♂	57	1	0.018
(<i>G. hirsutum</i> × <i>G. longicalyx</i>) ² ♂ × <i>G. hirsutum</i> ♀	6	2	0.3

Table 2. Ribbon width of the hexaploid (*G. hirsutum* × *G. longicalyx*)² and its parental species.

Genotype	Number of fiber analysed	Ribbon width (μm)		LSD grouping
		± standard deviation	Min Max	
<i>G. hirsutum</i> (cv. C2)	107	17.765 ± 0.207	12.092 24.369	C
<i>G. longicalyx</i>	113	5.940 ± 0.202	4.254 8.862	A
(<i>G. hirsutum</i> cv. C2 × <i>G. longicalyx</i>) ²	122	12.526 ± 0.180	8.946 16.008	B

G. longicalyx as reported by several authors [4] [12] [13], and its remarkable potential to improve the fiber fineness of *G. hirsutum*, with regard to the expression of this interesting trait in the hexaploid (*G. hirsutum* × *G. longicalyx*)².

The data concerning the evaluation of the resistance to the reniform nematode are presented in **Table 3**. The parental species *G. hirsutum* presented the greatest number of eggs per gram root (205.8 eggs/g root) while the number of eggs per gram root of *G. longicalyx* and the hexaploid were very low, 0 and 3.8 eggs per gram root respectively. Compared to the susceptible *G. hirsutum* species, *G. longicalyx* (0% eggs/g root) and the hexaploid (1.96% eggs/g root) were very resistant. This finding confirms the high resistance to the reniform nematode of the wild African cotton species *G. longicalyx* [14] [15] and shows the inheritance and expression of this interesting trait in the hexaploid.

3.6. Molecular Analysis with AFLP Markers

The results of the AFLP analysis are presented in **Table 4**. The AFLP electrophoretic profile obtained (**Figure 4**) revealed a total of 143 loci distributed in 52, 45 and 46 loci for respectively the primer pairs E-ACC/M-CAG, E-ACT/M-CTG and E-ACT/M-CAG. Of the 143 loci revealed, 57 were common to *G. hirsutum* and *G. longicalyx* and therefore monomorphic between these two parental species. All of these 57 monomorphic loci were detected in the hexaploid. In addition to the monomorphic loci, 44 loci were specific to *G. hirsutum* and 42 others were specific to *G. longicalyx*, which makes 86 polymorphic loci in all, representing a percentage of polymorphism of 60.14%. Thus, the AFLP markers

Table 3. Results of the assessment of the resistance to the reniform nematode of the hexaploid (*G. hirsutum* × *G. longicalyx*)² and its parental species.

Genotypes	No of Plants	Eggs No per gram root	Percentage of egg per gram root compared to <i>G. hirsutum</i> control	Host status
<i>G. hirsutum</i> (cv C2)	5	205.8 ± 7.63	100%	S ^a
<i>G. longicalyx</i>	5	0	0%	HR ^b
Hexaploid	30	3.8 ± 3.11	1.96% ± 1.6	HR

^aS: susceptible; ^bHR: highly resistant.

Table 4. Results of the analysis of the AFLP electrophoretic profile of the hexaploid (*G. hirsutum* × *G. longicalyx*)² and its parental species.

AFLP primer pairs	Total No of bands generated	Total No of <i>G. hirsutum</i> bands	No of <i>G. hirsutum</i> specific (non specific) bands	No of <i>G. hirsutum</i> specific bands present (absent) in the hexaploid	Total No of <i>G. longicalyx</i> bands	No of <i>G. longicalyx</i> specific (non specific) bands	No of <i>G. longicalyx</i> specific bands present (absent) in the hexaploid	Total No of bands in the hexaploid
E-ACC/M-CAG	52	34	10 (24)	10 (0)	42	18 (24)	16 (2)	50
E-ACT/M-CTG	45	36	21 (15)	21 (0)	24	9 (15)	9 (0)	45
E-ACT/M-CAG	46	31	13 (18)	13 (0)	33	15 (18)	13 (2)	44
Total	143	101	44 (57)	44 (0)	99	42 (57)	38 (4)	139

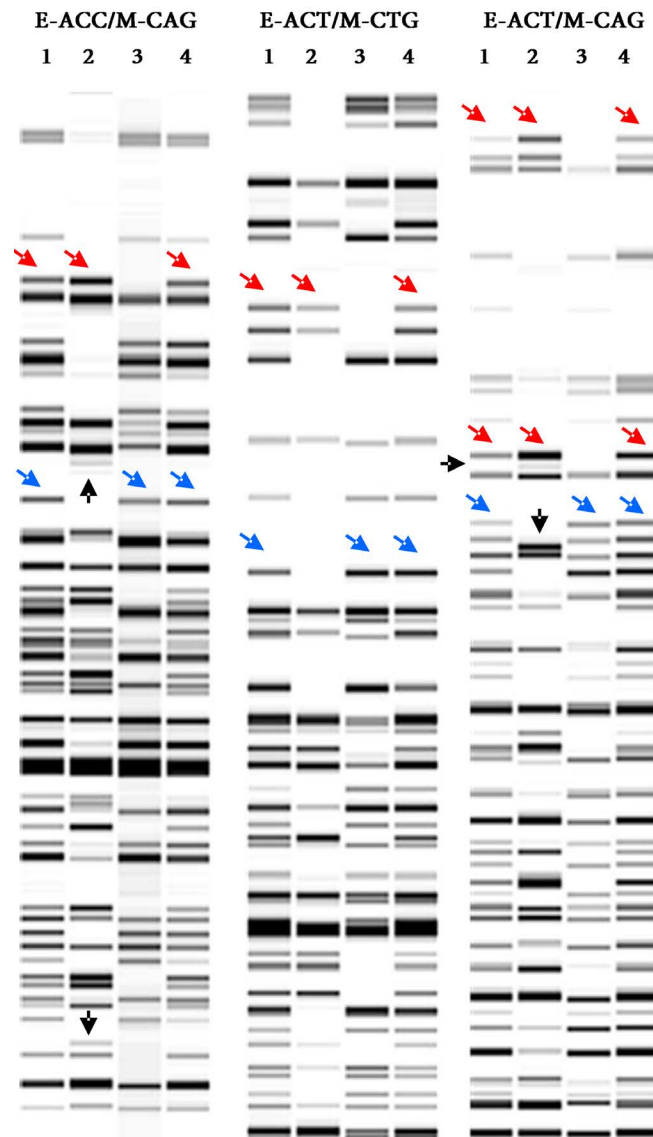


Figure 4. AFLP electrophoretic profile of the hexaploid (*G. hirsutum* × *G. longicalyx*)² and its parental species using the three primer pairs E-ACC/M-CAG, E-ACT/M-CTG and E-ACT/M-CAG: 1 = hexaploid#1; 2 = *G. longicalyx*; 3 = *G. hirsutum*; 4 = hexaploid#2. The red arrows show some *G. longicalyx* specific bands; the blue arrows show some *G. hirsutum* specific bands; the black arrows show specific bands *G. longicalyx* that are missing in the hexaploid hybrid.

efficiently discerned differences between the two parental species and distinguished them distinctly from each other. This is consistent with [38] who also highlighted such differences between *G. hirsutum* and *G. longicalyx*, using SNP markers.

All 44 specific loci of *G. hirsutum* and 38 of 42 specific loci of *G. longicalyx* were found in the hexaploid. In total, all the loci revealed in the hexaploid come from *G. hirsutum* and *G. longicalyx*, which confirms the hybrid status of the hexaploid as indicated by cytological analysis. However, 4 of the 42 specific loci

of *G. longicalyx* were missing in the hexaploid (Figure 4). [20] studying the synthetic allohexaploid (*G. hirsutum* × *G. anomalum*)² also found missing SSR alleles of the wild species *G. anomalum* in the hexaploid. Generally, the main reasons used to explain parental band missings in hybrids are loss of chromosomes or rearrangements of chromosomes. In the present study, the loss of chromosomes cannot explain the missing AFLP loci because the synthetic allohexaploid had the expected number of chromosomes (2n = 78). The explanation of chromosomal rearrangements is also unlikely since the two different hexaploid plants used for the molecular analysis had exactly the same missing loci. It is unlikely that there will be exactly the same recombination in two different plants. A slight genetic differentiation between the *G. longicalyx* plant used to develop the hexaploid hybrid and the *G. longicalyx* plant used in this molecular analysis could likely be the reason for the missing loci in the hexaploid hybrid. Indeed, intraspecific differentiation exists in wild cotton species. For example, [39] using AFLP data, reported intraspecific variation (in terms of percentage of polymorphic fragments) in four species of cotton (*G. aridum*, *G. laxum*, *G. lobatum*, and *G. schwendimani*). Anyway, phenotypic analysis showed that the current synthetic allohexaploid hybrid had the characteristics of interest (resistance to reniform nematode and fineness of the fibers) sought in *G. longicalyx*. Therefore, this hexaploid material is perfectly suited for use in a cotton improvement program.

4. Conclusion

The present study demonstrated the hybridity and hexaploid status of the genotype studied using cytological and molecular marker methods. Moreover, this synthetic allohexaploid hybrid exhibited the useful traits of the African wild diploid species *G. longicalyx* with regard to the fineness of the fibers and the resistance to reniform nematode. This synthetic allohexaploid hybrid represents very interesting genetic stocks that can be used as a bridge for the transfer of useful agronomic traits from wild species to upland cotton varieties.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

- [1] Ayubov, M.S., Abdurakhmonov, I.Y., Sripathi, V.R., Saha, S., Norov, T.M., Buriev, Z.T., Shermatov, S.E., Ubaydullaeva, K.A., McCarty, J.C., Deng, D.D. and Jenkins, J.N. (2018) Recent Developments in Fiber Genomics of Tetraploid Cotton Species. Intechopen, London. <https://doi.org/10.5772/intechopen.72922>
- [2] Fang, L., Gong, H., Hu, Y., Liu, C., Zhou, B., Huang, T., *et al.* (2017) Genomic Insights into Divergence and Dual Domestication of Cultivated Allotetraploid Cottons. *Genome Biology*, **18**, 33. <https://doi.org/10.1186/s13059-017-1167-5>
- [3] Wu, Y., Liu, F., Yang, D.G., Li, W., Zhou, X.J., Xiao, Y.P., *et al.* (2018) Comparative Chloroplast Genomics of *Gossypium* Species: Insights into Repeat Sequence Varia-

- tions and Phylogeny. *Frontiers in Plant Science*, **9**, 376.
<https://doi.org/10.3389/fpls.2018.00376>
- [4] Konan, N.O. and Mergeai, G. (2020) Relationship between Meiotic Behaviour and Fertility in Backcross-1 Derivatives of the [(*Gossypium hirsutum* × *G. thurberi*)² × *G. longicalyx*] Trispecies Hybrid. *Comparative Cytogenetics*, **14**, 75-95.
<https://doi.org/10.3897/CompCytogen.v14i1.47231>
- [5] Wendel, J.F. and Grover, C.E. (2015) Taxonomy and Evolution of the Cotton Genus, *Gossypium*. In: Fang, D.D. and Percy, R.G., Eds., *Cotton*, American Society of Agronomy Inc., Madison, 25-44. <https://doi.org/10.2134/agronmonogr57.2013.0020>
- [6] Gallagher, J.P., Grover, C.E., Rex, K., Moran, M. and Wendel, J.F. (2017) A New Species of Cotton from Wake Atoll, *Gossypium stephensii* (Malvaceae). *Systematic Botany*, **42**, 115-123. <https://doi.org/10.1600/036364417X694593>
- [7] Wang, K., Wendel, J.F. and Hua, J. (2018) Designations for Individual Genomes and Chromosomes in *Gossypium*. *Journal of Cotton Research*, **1**, 3.
<https://doi.org/10.1186/s42397-018-0002-1>
- [8] Ijaz, B., Zhao, N., Kong, J. and Hua, J. (2019) Fiber Quality Improvement in Upland Cotton (*Gossypium hirsutum* L.): Quantitative Trait Loci Mapping and Marker Assisted Selection Application. *Frontiers in Plant Science*, **10**, 1585.
<https://doi.org/10.3389/fpls.2019.01585>
- [9] Shim, J., Mangat, P.K. and Angeles-Shim, R.B. (2018) Natural Variation in Wild *Gossypium* Species as a Tool to Broaden the Genetic Base of Cultivated Cotton. *Journal of Plant Science: Current Research*, **2**, 005.
- [10] Islam, M.S., Zeng, L., Thyssen, G.N., Delhom, C.D., Kim, H.J., Li, P. and Fang, D.D. (2016) Mapping by Sequencing in Cotton (*Gossypium hirsutum*) Line MD52ne Identified Candidate Genes for Fiber Strength and Its Related Quality Attributes. *Theoretical and Applied Genetics*, **129**, 1-6.
<https://doi.org/10.1007/s00122-016-2684-4>
- [11] Mergeai, G. (2006) Interspecific Introgressions in Cotton. *Cahiers Agricultures*, **15**, 135-143.
- [12] Gotmare, V., Singh, P. and Tule, B.N. (2000) Wild and Cultivated Species of Cotton. CICR Technical Bulletin No. 5, CICR, Mumbai, 1-21.
- [13] Nacoulima, L.N., Diou, F.H., Konan, N.O. and Mergeai, G. (2016) Production of New Cotton Interspecific Hybrids with Enhanced Fiber Fineness. *Journal of Agricultural Science*, **8**, 46-56. <https://doi.org/10.5539/jas.v8n2p46>
- [14] Yik, C.P. and Birchfield, W. (1984) Resistant Germplasm in *Gossypium* Species and Related Plants to *Rotylenchulus reniformis*. *Journal of Nematology*, **16**, 146-153.
- [15] Konan, N.O., De Proft, M., Ruano, O., Baudoin, J.-P. and Mergeai, G. (2014) A Screening Procedure for Evaluating Cotton for *Rotylenchulus reniformis* Resistance in Controlled Conditions. *Tropicicultura*, **32**, 3-9.
- [16] Konan, N.O. and Mergeai, G. (2007) Breeding Possibilities of the Main Cultivated Cotton Species (*Gossypium hirsutum* L.) for the Resistance to Reniform Nematode (*Rotylenchulus reniformis* Linford et Oliveira). *Biotechnology, Agronomy, Society and Environment*, **11**, 159-171.
- [17] Sarr, D. and Mergeai, G. (2009) Genetic Broadening of the Main Cultivated Cotton Species *Gossypium hirsutum* L. by Creation and Exploitation of Monosomic Alien Addition Lines. *Biotechnology, Agronomy, Society and Environment*, **13**, 187-201.
- [18] Konan, N.O., D'Hont, A., Baudoin, J.P. and Mergeai, G. (2007) Cytogenetics of a New Trispecies Hybrid in Cotton: [(*Gossypium hirsutum* L. × *G. thurberi* Tod.)² ×

- G. longicalyx* Hutch. & Lee]. *Plant Breeding*, **126**, 176-181.
<https://doi.org/10.1111/j.1439-0523.2007.01325.x>
- [19] Mergeai, G. (2004) Forty Years of Genetic Improvement of Cotton through Interspecific Hybridisation at Gembloux Agricultural University: Achievement and Prospects. *Proceedings of the World Cotton Research Conference*, Cape Town, 9-13 March 2003, 120-133.
- [20] Zhang, X., Zhai, C., He, L., Guo, Q., Zhang, X., Xu, P., *et al.* (2014) Morphological, Cytological and Molecular Analyses of a Synthetic Hexaploid Derived from an Interspecific Hybrid between *Gossypium hirsutum* and *Gossypium anomalum*. *The Crop Journal*, **2**, 272-277. <https://doi.org/10.1016/j.cj.2014.06.009>
- [21] Roehrich, O. (1947) General Method for the Study of Technological Characteristics of Plant Textile Fibers. *Coton et Fibres Tropicales*, **2**, 81-89.
- [22] Vroh Bi, I., Baudoin, J.-P., Hau, B. and Mergeai, G. (1999) Development of High-Gossypol Cotton Plants with Low Gossypol Seeds Using Trispecies Bridge Crosses and *in Vitro* Culture of Seed Embryos. *Euphytica*, **106**, 243-251.
<https://doi.org/10.1023/A:1003539924238>
- [23] Dhooghe, E., Van-Laere, K., Eeckhaut, T., Leus, L. and Van-Huylenbroeck, J. (2011) Mitotic Chromosome Doubling of Plant Tissues *in Vitro*. *Plant Cell Tissue and Organ Culture*, **104**, 359-373. <https://doi.org/10.1007/s11240-010-9786-5>
- [24] Cohen, H., Fait, A. and Tel-Zur, N. (2013) Morphological, Cytological and Metabolic Consequences of Autopolyploidization in *Hylocereus* (Cactaceae) Species. *BMC Plant Biology*, **13**, Article No. 173.
<https://www.biomedcentral.com/1471-2229/13/173>
<https://doi.org/10.1186/1471-2229-13-173>
- [25] Castro, M., Castro, S. and Loureiro, J. (2018) Production of Synthetic Tetraploids as a Tool for Polyploid Research. *Web Ecology*, **18**, 129-141.
<https://doi.org/10.5194/we-18-129-2018>
- [26] Beest, M., Le Roux, J.J., Richardson, D.M., Brysting, A.K., Suda, J., Kubesová, M. and Pysek, P. (2012) The More the Better? The Role of Polyploidy in Facilitating Plant Invasions. *Annals of Botany*, **109**, 19-45. <https://doi.org/10.1093/aob/mcr277>
- [27] Hosseini, H., Chehrazi, M., Sorestani, M. and Ahmadi, D. (2013) Polyploidy and Comparison of Diploid and Autotetraploid Seedling of Madagascar Periwinkle (*Catharanthus roseus* cv. alba). *International Research Journal of Applied and Basic Sciences*, **4**, 402-406.
- [28] Chae, W.B., Hong, S.J., Gifford, J.M., Lane Rayburn, A., Widholm, J.M. and Juvik, J.A. (2013) Synthetic Polyploid Production of *Miscanthus sacchariflorus*, *Miscanthus sinensis*, and *Miscanthus x giganteus*. *GCB Bioenergy*, **5**, 338-350.
<https://doi.org/10.1111/j.1757-1707.2012.01206.x>
- [29] Münzbergová, Z. (2017) Colchicine Application Significantly Affects Plant Performance in the Second Generation of Synthetic Polyploids and Its Effects Vary between Populations. *Annals of Botany*, **120**, 329-339.
<https://doi.org/10.1093/aob/mcx070>
- [30] Liu, B. and Wendel, J.F. (2003) Epigenetic Phenomena and the Evolution of Plant Allopolyploids. *Molecular Phylogenetics and Evolution*, **29**, 365-379.
[https://doi.org/10.1016/S1055-7903\(03\)00213-6](https://doi.org/10.1016/S1055-7903(03)00213-6)
- [31] Doyle, J.J., Flagel, L.E., Paterson, A.H., Rapp, R.A., Soltis, D.E., Soltis, P.S. and Wendel, J.F. (2008) Evolutionary Genetics of Genome Merger and Doubling in Plants. *Annual Review of Genetics*, **42**, 443-461.
<https://doi.org/10.1146/annurev.genet.42.110807.091524>

- [32] Padmanaban, S., Zhang, P., Hare, R.A., Sutherland, M.W. and Martin, A. (2017) Pentaploid Wheat Hybrids: Applications, Characterisation, and Challenges. *Frontiers in Plant Science*, **8**, 358. <https://doi.org/10.3389/fpls.2017.00358>
- [33] Chu, C.-G., Faris, J., Friesen, T. and Xu, S. (2006) Molecular Mapping of Hybrid Necrosis Genes *Ne1* and *Ne2* in Hexaploid Wheat Using Microsatellite Markers. *Theoretical and Applied Genetics*, **112**, 1374-1381. <https://doi.org/10.1007/s00122-006-0239-9>
- [34] Rieseberg, L.H., Kim, S., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C. and Clay, K. (2007) Hybridization and the Colonization of Novel Habitats by Annual Sunflowers. *Genetica*, **129**, 149-165. <https://doi.org/10.1007/s10709-006-9011-y>
- [35] López-Caamal, A. and Tovar-Sánchez, E. (2014) Genetic, Morphological, and Chemical Patterns of Plant Hybridization. *Revista Chilena de Historia Natural*, **87**, 16. <https://www.revchilhistnat.com/content/87/1/16>
<https://doi.org/10.1186/s40693-014-0016-0>
- [36] Mehetre, S.S., Gawande, V.L. and Aher, A.R. (2002) Incompatibility in Wide Hybridization of *Gossypium* Species: Causes and Remedies—A Review. *Journal of Cotton Research and Development*, **16**, 111-124.
- [37] Tonosaki, K., Osabe, K., Kawanabe, T. and Fujimoto, R. (2016) The Importance of Reproductive Barriers and The Effect of Allopolyploidization on Crop Breeding. *Breeding Science*, **66**, 333-349. <https://doi.org/10.1270/jsbbs.15114>
- [38] Hinze, L.L., Hulse-Kemp, A.M., Wilson, I.W., *et al.* (2017) Diversity Analysis of Cotton (*Gossypium hirsutum* L.) Germplasm Using the CottonSNP63K Array. *BMC Plant Biology*, **17**, Article No. 37. <https://doi.org/10.1186/s12870-017-0981-y>
- [39] Álvarez, I. and Wendel, J.F. (2006) Cryptic Interspecific Introgression and Genetic Differentiation within *Gossypium aridum* (Malvaceae) and Its Relatives. *Evolution*, **60**, 505-517. <https://www.jstor.org/stable/4095313>
<https://doi.org/10.1111/j.0014-3820.2006.tb01132.x>



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