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Genotype-by-Environment Interaction and Stability of Canola (*Brassica napus* L.) for Weed Suppression through Improved Interference

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Abstract: Canola (*Brassica napus* L.) is a profitable grain crop for Australian growers. However, weeds remain a major constraint for its production. Chemical herbicides are used for weed control, but this tactic also leads to the evolution of herbicide resistance in different weed species. The suppression of weeds by crop interference (competition and allelopathic) mechanisms has been receiving significant attention. Here, the weed suppressive ability and associated functional traits and stability of four selected canola genotypes (PAK85388-502, AV-OPAL, AV-GARNET, and BAROSSA) were examined at different locations in NSW, Australia. The results showed that there were significant effects of canola genotypes and of genotypes by crop density interaction on weed growth. Among the tested genotypes, PAK85388-502 and AV-OPAL were the most weed suppressive and, at a plant density of 10 plants/m², they reduced the weed biomass of wild radish, shepherd's purse, and annual ryegrass by more than 80%. No significant differences were found in the primary root lengths among canola varieties; however, plants of the most weed-suppressive genotype PAK8538-502 exhibited a 35% increase in lateral root number relative to plants of the less weed-suppressive genotype BAROSSA. The analysis of variance revealed a significant influence of genotypes with PAK85388-502 and AV-OPAL performing the best across all the research sites. Results showed that canola genotypes PAK85388-502 and AV-OPAL were more weed suppressive than AV-GARNET and BAROSSA and may release specific bioactive compounds in their surroundings to suppress neighboring weeds. This study provides valuable information that could be utilised in breeding programs to select weed-suppressive varieties of canola in Australia. Thus, lateral root number could be a potential target trait for weed-suppressive varieties. Additionally, other root architecture traits may contribute to the underground allelopathic interaction to provide a competitive advantage to the crop.

Keywords: competition; neighbouring plant; root; root exudates; adaption



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1. Introduction

Canola (*Brassica napus* L.) is an oil seed crop belonging to the Brassicaceae family. It results from the natural hybridisation between *Brassica oleracea* L. and *Brassica rapa* L. Australia is the world's second-largest exporter of canola [1]. Canola grown in Australia meets the high expectations of exporters, domestic crushers, and intensive livestock producers. However, weeds are an important biotic constraint on canola production, resulting in yield and quality losses [2,3]. Grass weeds such as annual ryegrass (*Lolium rigidum*), vulpia (*Vulpia myuros*), and wild oat (*Avena fatua*) are the most abundant weed species in canola crops of south-eastern Australia [4]. Weeds from Brassicaceae can contaminate

canola seed samples, which leads to increased levels of erucic acid and glucosinolates with the consequent reduction in canola quality due to their contamination [2–5].

Chemical herbicides and mechanical weeding have been the most frequently used methods to control weeds [6,7] and have served to keep weed infestations low, thereby improving crop productivity. Despite the significant contribution of these methods, there are also certain associated challenges. Mechanical weed control requires extra soil turnover, which can disturb soil structure and deplete soil fertility [8]. Additionally, it is not always effective and can be expensive and lack durability [9]. Similarly, herbicide-resistant weeds, health effects, and environmental concerns are the major constraints for repeated use of herbicides [10,11]. In Australia, non-chemical weed control options are highly sought after for incorporation into improved integrated weed management systems (IWMS) to overcome herbicide resistance.

Crop plant interference against weeds involves the combined effects of plant competition and allelopathy. Competition is the negative interaction between two or more plant species for resources within a limited space and is distinct from plant allelopathic interaction [12]. The competitiveness of a plant is influenced by various morphological, phenological, and agronomic traits [13–15]. Increasing crop seeding rates is one of the simplest agronomic ways to enhance crop competitiveness [16], as it increases crop canopy development and hastens nutrient use, thereby denying these resources to weeds and thus reducing weed pressure [17,18]. In contrast to competition, allelopathy is the exudation of compounds by plant roots that can suppress the growth of neighbouring plants and affect seeds and seedlings of other species located within a limited range [19]. Although most plant species, including crops, can produce and release biologically active root exudates (allelochemicals), relatively few have strong allelopathic properties. Several bioactive compounds were isolated from strongly allelopathic canola genotypes such as AV-OPAL and PAK85388-502 [20]. These phytotoxic or signalling chemicals presumably resulted in the observed inhibitory effects on annual ryegrass (*L. rigidum*) under laboratory conditions and may also be responsible for the significant suppression of other weed species in the field [20]. Allelochemical concentrations are a function of the density of the allelopathic crop [21,22]. This suggests that density may be an important factor in enhancing canola allelopathic activity. Recent research further highlighted that crop performance against weeds can be improved by taking account both competitiveness and allelopathy. Assessing crop allelopathy in the field is challenging; however, research work is beginning in this direction [23,24].

Crop species and genotypes within the species differ in root traits and their ability to compete for below-ground resources, which can in turn influence aboveground traits and yield [25,26]. Root and shoot architecture including length, biomass, number of lateral roots, and growth play a crucial role in competition and crop performance [27–30]. Hence, screening crop varieties with plant functional traits may offer new insights into plant allelopathy. Furthermore, plants can perceive different external and internal signals from their surroundings, while changes in environmental conditions can subsequently affect plant allelopathy and its functional traits [31,32]. Huang et al. [33] demonstrated that *Merremia boissiana* can adjust its resource allocation to allelopathy and leaf functional traits to adapt to varying environments. As one of the key factors affecting plant allelopathy and functional traits, environmental changes have long been assumed to be critical. However, the link between canola allelopathy and its functional traits has received little attention.

Understanding how crop genotypes respond differently to changing environmental conditions is crucial and it is a significant step in developing improved crop varieties [33]. When genotypes are assessed across various locations or years, their yield and individual traits' performances may vary significantly. The presence of substantial genotype-by-environment ($G \times E$) interactions can further complicate comparisons and recommendations for adaptable genotypes [34]. To identify stable genotypes, it is essential to break down the $G \times E$ interaction into stability statistics assigned to each genotype across different environments. Various stability indices have enabled researchers to pinpoint widely

adapted genotypes for breeding programs or enhance recommendations to growers [35]. A genotype is considered most stable when it exhibits minimal fluctuation across diverse environments [36]. The present research aimed to examine (1) the functional traits and weed-suppressive ability of four selected canola genotypes and (2) the stability of canola genotypes for weed suppression over different temporal and spatial conditions.

2. Materials and Methods

2.1. Experimental Site

Three field sites were chosen in southern New South Wales (NSW) in 2016, one each at Wagga Wagga Agricultural Institute (WWAI) (−35.04591 E, 147.3676 S), Temora (−34.3779 E, 147.4878 S), and Marrar (−34.8270 E, 147.3528 S) of NSW in Australia, respectively. Prior to that, a field study was conducted in 2013 at WWAI [2]. The Wagga Wagga sites had a high population of *L. rigidum* (annual ryegrass) (80 plants/m²) and the Marrar site was severely affected by *Raphanus raphanistrum* (wild radish).

2.2. Canola Genotypes and Sowing Density

Four canola genotypes, namely PAK85388-502, AV-OPAL, AV-GARNET, and BAROSSA, were selected, based on a previous study by Asaduzzaman et al. [2,37]. The genotypes are open-pollinated; PAK85388-502 is a breeding line and AV-GARNET is a competitive cultivar reported by Lemerle et al. [38]. AV-OPAL and PAK85388-502 were categorised as allelopathic canola genotypes [20]. A previous laboratory experiment with several canola genotypes showed that canola densities played a major role in its allelopathic activity in suppressing annual ryegrass root growth [5]. Hence, for each genotype, four different sowing rates (15, 30, 60, and 120 seeds/m²) were used in 1.8 m × 10 m plots with 4, 5, and 5 replications for Marrar 2016, Temora 2016, and Wagga Wagga 2016 sites, respectively. The Wagga Wagga 2013 field site had 6 replications [39]. The purpose was to determine canola density effects on weed growth and reproductive development.

2.3. Pre-Sowing Knockdown Herbicide and Sowing

Glyphosate (450 g/L Glyphosate) at 2 L/ha was applied at all sites as a pre-planting approach and no other herbicides were used during the experiment. The crop was sown with a plot seeder together with a basal fertilizer of Croplift 15 (Incitec Pivot Fertilisers™) at 120 kg/ha. This basal fertilizer was applied below the canola seed at sowing and canola seeds were treated with Jockey® (167 g/L fluquinconazole) at the recommended rate (2 L/100 kg seed) to control blackleg (*Leptosphaeria maculans*).

2.4. Data Collection to Assess Weed Suppressive Ability of Canola Genotypes

Weed numbers and biomass (as an indicator of weed seed bank replenishment), botanical compositions of weeds with respective genotypes, and shoot and root architecture were considered the most important metrics for allelopathic and interference effects in Wagga Wagga 2016 and Marrar 2016 sites (Table 1). Weed numbers were assessed using two quadrats (0.5 m × 0.5 m) per plot at the Wagga Wagga 2013, 2016, and Marrar 2016 sites. Quadrats were placed at random within each plot, but obvious weed patches were avoided. Weed biomass assessment was undertaken using the two quadrats per plot when canola biomass was estimated to be maximal, corresponding with early flowering in canola at Wagga Wagga 2013 and 2016, Temora 2016, and Marrar 2016 sites. Canola root and shoot measurements were taken just prior to biomass cut by measuring 20 random plants per genotype at Wagga Wagga 2016 and Marrar 2016 sites. A total of 20 plants were randomly selected and carefully dug up and the lengths of the canola root and shoot were measured. Then, plants were transported to a shaded area of WWAI where the number of lateral roots was counted. The relative root growth (RRG) of 20 plants for each genotype was calculated as follows: $RR_{YP} = R_{ij}/R_{ii}$, where R_{ii} is the canola root biomass in the weeds-free condition and R_{ij} is the biomass of canola roots collected from heavily weed-infested plots. Leaf Area Index (LAI) and Photosynthetic Active Radiation (PAR) were calculated based

on Ceptometer data, where Transmitted PAR = Below-canopyPAR/ Above-canopy PAR. The NDVI and Ceptometer readings were measured from the treatment plots at 60 plant density/m² for each genotype, just before canola flower initiation.

Table 1. Data measurements for four different field experiments.

Name	Units	Where Measured	Site
Weed number	plants/m ²	2 × quads (0.5 m × 0.5 m per plot)	Wagga Wagga 2016 and 2013 and Temora 2016
Weed biomass (drymatter)	g/m ²	2 × quads (0.5 m × 0.5 m per plot)	Wagga Wagga 2016 and 2013 and Temora 2016
NDVI (greenness index)	unit less	Whole plot length	Wagga Wagga 2016 and Marrar 2016
Ceptometer (LAI and PAR)	unit less	Whole plot length	Wagga Wagga 2016 and Marrar 2016
Canola root length, lateral roots and relative root growth	cm, no./plant	20 plants/genotype	Wagga Wagga 2016 and Marrar 2016
Canola shoot length, diameter and stem-specific density	cm, g cm ⁻³	20 plants/genotype	Wagga Wagga 2016 and Marrar 2016

Note: Weed counting and weed biomass (dry matter) were measured in two 0.5 m × 0.5 m quadrants per plot in Wagga Wagga (2016 and 2013) and Temora (2016).

The Stem-Specific Density (SSD) (g cm⁻³ or kg dm⁻³) was measured at canola flowering time. Briefly, the main stem oven-dry mass (at 70 °C for 72 h) of a canola plant was measured and divided by the volume of the same section. Where the volume of a canola stem was determined simply by measuring its total length and its diameter in three places along the freshly harvested stem using calipers, SSD is emerging as a core functional trait because of its importance for the stability, defence, architecture, C gain, and growth potential of plants. A low stem density (with large vessels) leads to fast growth, whereas a high stem density (with small vessels) leads to a high survival, because of biomechanical and hydraulic safety [38].

2.5. Statistical Analysis

The field experiments were conducted with randomised complete block designs. The broad-leaf weed numbers were not analysed separately but all broad-leaf weeds were included in the weed biomass calculations. Weed biomass production in response to different crop densities were analysed in the linear regression model. A multivariate correlation matrix analysis was conducted to see the relations among the observed variables. To identify the most successful genotype for weed suppression within each environmental effect, R packages including *metan* [40] were used intensively for data analysis. A joint ANOVA was performed by using the following formula.

$$y_{ijk} = \mu + \alpha_i + \tau_j + (\alpha\tau)_{ij} + \gamma_{jk} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the response variable (e.g., weed biomass) observed in the k th block of the i th genotype in the j th environment ($i = 1, 2, \dots, g; j = 1, 2, \dots, e; k = 1, 2, \dots, b$); μ is the grand mean; α_i is the effect of the i th genotype; τ_j is the effect of the j th environment; $(\alpha\tau)_{ij}$ is the interaction effect of the i th genotype with the j th environment; γ_{jk} is the effect of the k th block within the j th environment; and ε_{ijk} is the random error. The stability analysis was conducted using ANOVA, incorporating the non-parametric Shukla's stability variance parameter [41] to assess the general superiority of genotypes across different locations. Additionally, the commonly used regression model proposed by Eberhart and Russell [42] for stability analysis of genotypes was employed where the dependent variable is predicted as a function of an environmental index, according to the following model:

$$Y_{ij} = \beta_{0i} + \beta_{1ij} + \delta_{ij} + \varepsilon_{ij} \quad (2)$$

where β_{0i} is the grand mean of the genotype i ($i = 1, 2, \dots, I$); β_{1i} is the linear response (slope) of the genotype i to the environmental index I_j ($j = 1, 2, \dots, e$); δ_{ij} is the deviation from the regression line for the i -th genotype in the j -th environment, indicating the specific interaction between genotype and environment; and ε_{ij} is the random error. Finally, the additive main effects and multiplicative interaction (AMMI) biplot was used ($G \times E$) to visually represent genotype performances and environmental interactions on weed biomass production.

3. Results

3.1. Canola and Weed Biomass

The composition of weed species differed between sites. Trial sites at Temora and Wagga Wagga 2013 and 2016 were mainly infested by annual ryegrass and, at the early seedling stage of canola, there was no significant ($p > 0.005$) difference between genotypes in annual ryegrass densities. The Marrar site was affected mostly by wild radish [WR] followed by shepherd's purse [SP], annual ryegrass [ARG], and other broadleaf weeds [BW]. Also, the total weed densities [WR+SP+ARG+BW] at the early growth stage of canola were not significantly ($p > 0.05$) different between genotypes. There was a significant ($p < 0.001$) interaction effect between canola density and genotype on total weed biomass (Figure 1). A significant negative correlation was observed between weed biomass and canola densities (Figure 1). At 10 canola plants/ m^2 , the total weed dry biomass was only 17 g and 10 g/ m^2 for the genotype PAK85388-502 and 25 g and 22 g for AV-OPAL at Wagga Wagga 2016 and Marrar 2016 sites, respectively. Genotype BAROSSA was less suppressive on weed growth; this genotype, even at high density (10 canola plants/ m^2), produced double the weed biomass (48 g) of AV-OPAL at the Wagga Wagga 2016 site.

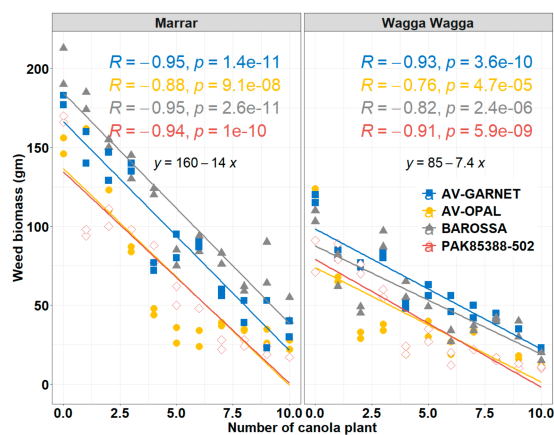


Figure 1. Negative linear relationship between canola plant density and weed biomass (m^2) with correlation coefficient values (R) and respective p -values. The data were collected from Marrar 2016 and Wagga Wagga 2016 experimental sites.

3.2. Plant Functional Traits

The multivariate pair analysis showed that canola root length was not significantly different among genotypes. Significant negative relationships between the number of lateral canola roots ($r = -0.18^*$) or specific canola stem density ($r = -0.19^*$) and weed biomass were observed (Figure 2). Genotype PAK85388-502 had the most lateral root/plant followed by AV-OPAL and Barossa, whereas AV-GARNET and BAROSSA had the most Relative Root Growth (RRG) in both experimental sites (Table 2). The stem density of canola did not differ significantly between genotypes ($p = 0.12$), environments ($p = 0.58$), and $G \times E$ ($p = 0.89$). Stem length ($p < 0.05$) and SSD ($p = 0.005$) were significantly different between genotypes where PAK85388-502 had the longest stem followed by AV-GARNET, while BAROSSA had the largest SSD value.

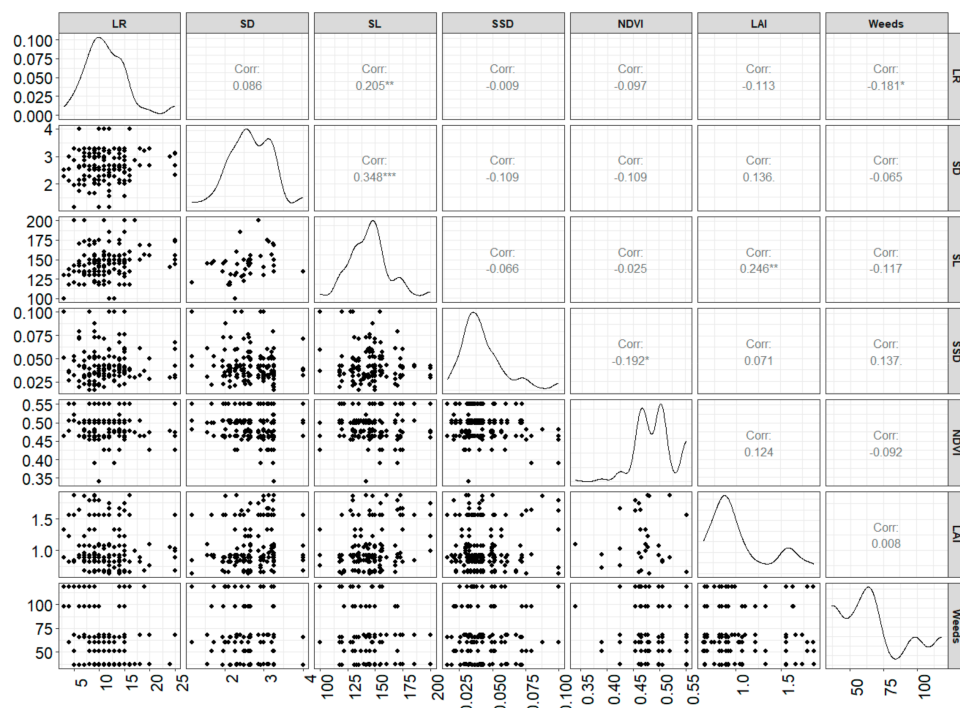


Figure 2. Correlation matrix among canola traits with weed biomass. Here, LR is the number of lateral roots/plants, SD is the stem density, SL is the stem length, SSD is the stem-specific density, NDVI is the normalised differential vegetation index, LAI is the leaf area index, and Weeds is total weed biomass. *, **, and *** indicate statistical significance at the 0.05; 0.01, and 0.001 level, respectively.

Table 2. Mean number of lateral roots (LR), relative root growth (RRG), shoot length (SL), stem specific density (SSD), NDVI, LAI, and PAR of four canola genotypes at Wagga Wagga (W) and Marrar sites (M) 2016.

Genotype	LR	RRG	SL (cm)	SSD	NDVI	LAI	PAR
AV-GARNET	9.80 (±0.63)	2.50 (±0.40) M 3.0 (±0.34) W	142.60 (±3.47)	0.04 (±0.002)	0.46 M 0.49 W	1.10	0.54
AV-OPAL	12.60 (±0.60)	1.10 (±0.19) M 1.80 (±0.24) W	137.60 (±4.00)	0.04 (±0.002)	0.49 M 0.46 W	1.00	0.53
BAROSSA	10.55 (±0.63)	3.18 (±0.49) M 2.00 (±0.21) W	136.73 (±6.50)	0.05 (±0.003)	0.45 M 0.49 W	1.20	0.50
PAK85388-502	14.35 (±0.58)	2.37 (±0.25) M 1.92 (±0.25) W	164.00 (±4.80)	0.03(±0.002)	0.47 M 0.51 W	1.10	0.48

The fractional ground cover (estimated by the NDVI) showed that genotype effects were not significant ($p = 0.06$). Additionally, the LAI and transmitted PAR were not significant ($p > 0.05$) among the genotypes (Table 2). Numerically, the highest leaf area (1.2) was produced by BAROSSA followed by the competitive and allelopathic genotypes AV-GARNET and PAK85388-502, respectively. The light interception capability was higher in AV-OPAL (0.54) followed by AV-GARNET and PAK85388-502.

3.3. Environmental Effect and Stability of Genotypes for Weed Suppression

The interactions between genotypes and environments measure the spatial (locations) and temporal (years) separation and/or combination of these factors. The combined analysis of variance across environments showed that variances due to genotypes (G), environments (E), and $G \times E$ were highly significant ($p < 0.001$) for weeds biomass. So, it was appropriate to explore such interaction including ANOVA and linear regression-based

stability analysis. Table 3 shows that weed biomass was six and five times higher under unfavourable conditions than under favourable conditions for BAROSSA and AV-GARNET, respectively. For PAK85388-502 and AV-OPAL, weed biomass was five and four and a half times higher under unfavourable conditions compared with favorable conditions. The best weed suppressive genotypes for both favourable and unfavourable conditions were PAK85388-502 followed by AV-OPAL. Genotype BAROSSA was the least weed-suppressive in either condition. The regression model shows that slopes (b_1) of both AV-OPAL and PAK85388-502 were flatter than the other two varieties. The low coefficient (b_0) value and significant differences between these two genotypes (AV-OPAL and PAK85388-502) were due mostly to the genotypic effect rather than the environmental effect.

Table 3. Stability and adaptability of four canola genotypes for weed suppression at different environments and two different years.

Genotype	Analysis for all Environments		Analysis for Unfavourable Environments		Analysis for Favourable Environments		Shukla Stability	Regression Parameters			
	Weed biomass (g)	Rank	Weed biomass (g)	Rank	Weed biomass (g)	Rank		Rank	b_0	b_1	R^2
AV-GARNET	114	3	282	3	57.8	3	3	114	1.01	1.00	1.87
AV-OPAL	75.3	1	181	1	40.1	2	2	75.3	0.63	0.99	1.45
BAROSSA	155.0	4	420	4	65.9	4	4	155	1.58	0.99	4.15
PAK85388-502	81.3	2	212	2	37.7	1	1	81.3	0.78	0.99	2.42

Favourable environments: these are environmental conditions that are conducive to the optimal growth and development of the genotypes. Unfavorable environments: these conditions are less ideal for the genotypes, often causing stress or suboptimal growth. A low b_0 value suggests less variability observed in genotypes for suppression among environment conditions, while a low RMSE value indicates that the regression model fitted very well. The data range for weed biomass is $769 - 2 = 767$, and 1% of this range is 7.67, which is considered a threshold for a low RMSE value.

The top genotypes for weed suppression were PAK85388-502 and AV-OPAL at all four sites (Table 3 and Figure 3). The computed genotype-environment effects or genotype plus genotype-environment effects showed no clear change except Shukla ranking in the rank order of genotypes across environments. PAK85388-502 and AV-OPAL were the most stable genotypes for weed suppression across different environments.

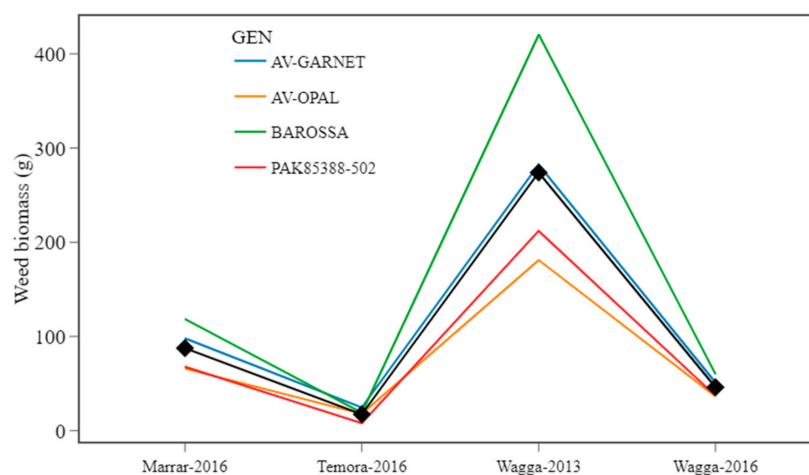


Figure 3. Performance and adaption of canola genotypes for weed suppression. The black solid line shows the average weed suppression at different locations.

Among the locations, the Temora 2015 site achieved the most weed suppression by the four canola genotypes, and they adapted very well compared to the other three sites (Figure 3). The Wagga 2013 site was the least weed-suppressive and the adaptability of the genotypes varied. The two weed-suppressive canola genotypes (AV-OPAL and PAK85388-502) tended to be clustered together. They were placed closer than the other two

canola genotypes (AV-GARNET and BAROSSA) and produced less weed biomass in their plots across the different locations. The Wagga 2013 site had the highest weed biomass.

4. Discussions

The study of plant interference is increasingly popular although the current systems of plant breeding largely ignore the abilities of a variety to exercise control over its weed challengers. The difficulty in studying interactions between plants is due to the complex nature of plant interference, defined as the combined effect of competition and allelopathy. However, the development of crops with the capability to exert allelopathic effects on weeds through root exudates is an attractive prospect [43]. Research has shown this potential in wheat [44], barley [45,46], rice [47–49], and canola [39]. In this study, genotypes such as AV-OPAL, PAK85388-502, AV-GARNET, and BAROSSA produced similar crop biomass but the genotypes PAK85388-502 and AV-OPAL tended to result in weed biomass lower than even the strongly competitive genotype (cv. AV-GARNET). Therefore, weed-suppressing genotypes have the potential for integrated weed management and further work is needed to produce a genotype with combined competitive ability and allelopathy. Bertholdsson [50] found that early weed biomass was significantly lower in the highly allelopathic wheat lines compared with the non-allelopathic lines. These wheat varieties produced lower yields, indicating that both yield and weed-suppressive allelopathic capability are independent traits [50]. Also, our research revealed that weed biomass was suppressed during the early growth stages of allelopathic canola genotypes, resulting in significantly less weed biomass harvested at the end of the experiment.

Four Brassica genotypes (AV-OPAL, AV-GARNET, BAROSSA, and PAK85388-502) showed similar patterns in the density–response curve for weed growth and there was a density by genotype interaction. This indicates that crop density plays a role in canola weed suppression. These results were consistent with previous research in rice [47,51] and in wheat [52]. The aboveground canopy of all tested genotypes had similar contributions to weed control. However, beyond that, an additional mechanism such as the allelopathy of AV-OPAL and PAK85388-502 might have played a role in their neighbouring weed suppression. AV-OPAL was identified as a less vigorous genotype with shorter plant height among other canola genotypes [2]. Asaduzzaman et al. [37] collected 70 international rapeseed varieties and evaluated their allelopathic potential by growing them in close proximity to *L. rigidum*. Rapeseed was sown at 10, 20, and 30 plants/m² against 15 plants/m² of annual ryegrass. Generally, the higher density of rapeseed resulted in higher suppression of *L. rigidum*. The varieties with strong allelopathic activity were PAK85388-502, AV-OPAL, BLN3343CO0402, and RIVETTE. Many of these genotypes, categorised as allelopathic, can release a range of allelochemicals through their root exudates of canola, with key chemicals being sinapyl alcohol, p-hydroxybenzoic acid, and 3,5,6,7,8-pentahydroxy flavones [20]. Furthermore, in Canada, canola competitiveness was improved by choice of variety and use of higher seeding rates [53,54]. The negative relationships between root (number of lateral roots) and shoot (stem density and stem length) functional traits and weed biomass suggest that these traits are important components for weed suppression by canola genotypes. Therefore, their simultaneous selection will be a good approach to increasing weed suppressive ability. This same relationship was observed by Afuape et al. [55]. Genetic variability is essential for selection [56]. However, a wide range of varieties should be evaluated at different locations to further verify such relationships.

The stability analysis aims at helping breeders identify which genotypes have specific and/or general adaptability to various production environments. Additionally, stability analysis helps determine the test environments for future evaluations of canola production with self-weeding capability. Three locations (excluding Wagga 2013) clustered in the same quadrants, indicating that these locations share similarities in terms of weed growth and canola genotype performance. The Temora environment and Wagga 2016 were higher-performing environments for weed suppression compared with others. This result means that testing data from one location can represent the performance of the same

materials in other similar locations. Therefore, conducting a stability analysis with a large set of canola genotypes will further help identify specific genotypes for both locations, as well as a stable genotype that can be cultivated across multiple locations, particularly locations that share similar attributes to the test locations. Understanding the environmental conditions in which canola varieties are tested is crucial for accurately interpreting results and extrapolating findings to diverse geographical locations or growing conditions. Highlighting higher-performing environments for weed suppression, such as the Temora environment and Wagga 2016, underscores the need for a comprehensive description of these locations' environmental characteristics. To address this gap, future research should prioritise conducting stability analyses with a diverse range of canola genotypes across various locations. This approach would enable breeders to identify specific genotypes suited for different environments and stable genotypes adaptable to multiple locations, facilitating informed decision making in canola allelopathic variety cultivation.

The weed-suppressive ability of a specific canola variety might be weed-specific and likely associated with a genetic as well as an environmental component of variance; breeding will be required for its maintenance [57]. Combining a variety's capability in reducing specific weed pressure with optimal agronomic practices that facilitate crop health will generally enhance cropping system sustainability and allow growers to extend the life of valuable herbicides [58]. However, the feasibility of using a variety for specific weed management may not be useful for economic outcomes because canola crops are infested naturally by a range of weed species [5]. However, a competitive variety of canola could be developed by incorporating traits such as increased lateral roots and stem length and density without compromising other desirable traits such as grain yield, quality, or disease resistance [14]. Canola weed suppressiveness can be optimised to reduce specific weed growth and reproduction through farming practices that allow the implementation of a variety of cultural techniques such as sowing crops with different planting dates to reduce other weed pressure [18]. In addition, the ability of crops to suppress weeds appears to be strongly variety-dependent [59–61].

Here, the experiments reported reinforce the need for the preservation of the older varieties such as AV-OPAL and PAK85388-502 (highly allelopathic) and AV-GARNET (highly competitive [15]) so that these benefits can be incorporated into new varieties. Also, this research highlights the need for new varieties to be evaluated for their interference capabilities in weedy field plots without herbicides. The information obtained in this future research will allow producers to broaden their armory against herbicide resistance by choosing weed-suppressive varieties, thereby helping manage herbicide-resistant weeds that threaten productivity, profitability, and food-security. The capability of crop variety to suppress weeds is being considered as a preferred criterion for cultivar selection in many parts of the world [62]. However, further research should focus on characterising crop competition in detail by measuring relevant traits, assessing allelopathic potential through controlled experiments by quantifying allelochemicals in the field, and employing multiple regression models to determine the impact of each trait on weed regulation [23,63]. The enhanced interference (competitive and allelopathic) potential of crop varieties can contribute to their self-weeding capabilities. By reducing weed infestation, self-weeding crops can enhance the effectiveness of other weed control methods and optimise resource utilisation.

5. Conclusions

The current study demonstrated considerable variation among the canola genotypes across the studied traits. The observed genotype-by-environment ($G \times E$) effects influenced the average ranks of genotypes in different environments, highlighting the necessity for multi-environment testing before making informed decisions. Furthermore, the $G \times E$ biplot analysis identified two distinct mega-environments (Wagga Wagga 2013 vs. others), indicating significant interactions between genotypes and environments that affect trait expression for evaluating canola genotypes in Australia and identified PAK85388-502 and AV-OPAL as the best-performing genotypes for weed suppression. These genotypes were

stable and adaptable across test environments and could be used as parental materials for further genetic improvement through plant breeding. BAROSSA and AV-GARNET were more competitive but might be less allelopathic and the other two varieties might be more allelopathic but less competitive. Therefore, two separate traits could potentially be combined through breeding programs. The current study determined the magnitude of genotype by environment interaction and stability for weed suppression ability of canola genotypes. Since most grain crop breeding programs are often tailored toward the development of high-yielding, biotic, and abiotic resistance and/or tolerance, this work has identified novel genotypes that could be used to breed weed-suppressive varieties in the future.

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