Growth behavior and glyphosate resistance level in 10 biotypes of *Echinochloa colona* in Australia

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Abstract

Recently, poor control of *Echinochloa colona* with glyphosate has been reported in no-till agriculture systems of the northern grain region (NGR) of Australia. Two experiments were conducted using 10 biotypes of *E. colona* selected from the NGR of Australia to understand differences in their growth behavior and resistance pattern. Growth studies revealed that these biotypes differed in plant height (53-70 cm plant\(^{-1}\)), tiller production (30-52 tillers plant\(^{-1}\)), leaf production (124-186 leaves plant\(^{-1}\)) and seed head production (37-65 seed heads plant\(^{-1}\)). Days taken to seed heads and shoot biomass in these biotypes ranged between 40-48 d and 21-27 g plant\(^{-1}\), respectively. Seed production in these biotypes ranged between 5380 and 10244 seeds plant\(^{-1}\); lowest for biotype B17/25 and highest for biotype B17/13. Correlation studies revealed that seed number plant\(^{-1}\) had a positive correlation with plant height (\(r = 0.67\)), tiller number plant\(^{-1}\) (\(r = 0.89\)), leaf number plant\(^{-1}\) (\(r = 0.73\)), seed heads plant\(^{-1}\) (\(r = 0.78\)), seed head weight (\(r = 0.79\)), shoot biomass (\(r = 0.77\)) and root biomass (\(r = 0.46\)). The glyphosate dose-response study showed a wide range of responses in these biotypes and the glyphosate dose required to reduce 50% biomass (GR\(_{50}\) values) was estimated between 217 to 2159 g a.e. glyphosate ha\(^{-1}\). GR\(_{50}\) values of biotypes B17/16, B17/34 and B17/35 were 719, 2159 and 884 g ha\(^{-1}\), respectively, making them 3, 10 and 4-fold resistant to glyphosate.
compared with the susceptible biotype B17/37. Growth behavior and seed production
potential in these biotypes had no correlation with the resistance index. These results suggest
that some biotypes of *E. colona* are highly problematic; for example, biotype B17/34 was not
only highly glyphosate-resistant, but also produced a high seed number (9300 seeds plant\(^{-1}\)).
This study demonstrated that there is a possibility of great risk with the increased use of
glyphosate for managing *E. colona* in the NGR of Australia. The results warrant integrated
weed management strategies and improved stewardship guidelines are required for managing
glyphosate-resistant biotypes of *E. colona* and to restrict further movement of resistant
biotypes to other regions of Australia.

**Keywords:** Barnyard grass, Herbicide dose, Junglerice, Phenology, Seed number, Weed
biomass

**Introduction**

*Echinochloa colona* (L.) Link (C\(_4\) plant) has emerged as a major weed in summer crops in
Australia and competes highly for water, sunlight and nutrients (1, 2). Worldwide, it is rated
among the 10 most troublesome weeds. *E. colona* is widely distributed in the northern grain
region (NGR) of Australia (3, 4, 5) and it costs Australian agriculture AU$ 14.7 million
annually(6). Therefore, it affects the economy of Australian agriculture enormously.

Emergence of multiple cohorts in the summer season, along with high capacity for
seed production and seed dispersal have allowed the spread of *E. colona* throughout the NGR
of Australia. The seeds remain viable in the soil for more than one year, causing continuous
recruitment (7). A significant portion of the fresh seeds of *E. colona* are dormant; therefore,
retention of viability of original seed dispersal caused continuous reinfestation year after
year.
In Australia, intraspecific variations in *E. colona* have been reported on the basis of genetic diversity (8). Such variations are referred to as clone, biotype or ecotype. Morphological studies of these biotypes may increase our knowledge further and identify how these biotypes adapt to climate change and play a role in invasiveness. A minor change in morphology or physiology of the plant may affect its adaptability in a changing climate and a large number of dispersed seeds in the field, combined with the ability of this weed to flower under a range of photoperiods, may contribute to its invasiveness (9).

In the NGR of Australia, *E. colona* is a very common weed in no-till fallow land and glyphosate spray is the most common management practice for managing this weed. Glyphosate was mostly used in orchards (high-value crops) when introduced in Australia during the 1970s, as it was relatively expensive (10). However, in the 1980s, its price declined, and its application became a common practice for weed control in a pre-seeding and fallow situation in Australia, which enabled the growers to adopt the conservation tillage practice. Glyphosate disrupts the shikimate pathways, reducing aromatic acid production via inhibition of the chloroplast enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Presently, control of some biotypes of *E. colona* in the NGR has become difficult with glyphosate as it has evolved resistance. The first case of glyphosate-resistant *E. colona* was reported in the NGR in 2007 (11). At present, 41 weed species have been reported as glyphosate-resistant worldwide (12). The evolved resistance may be due to intensive and repeated use of glyphosate (13, 14). *E. colona* has also evolved resistance to four other herbicide modes of action, in addition to glyphosate (15).

A better understanding of the differences between biotypes for control with glyphosate is essential for developing long-term strategies. Variation in growth, morphological and physiological characteristics may alter herbicide efficacy within a species.
Efficacy of glyphosate can be affected by plant species, biotype, plant development stage and environmental conditions (16).

Further, herbicide-resistant biotypes can spread from one area to another through pollen, seed or other propagules (17, 18). Therefore, it is important to understand characteristics of resistant biotypes of a specific area to make better decisions and long-term strategies for weed control (19, 20). Development of herbicide resistance in biotypes causes certain biochemical and physiological changes in the plant, which may be different from a susceptible biotype. A dose-response experiment is often conducted to assess the level of resistance in different biotypes. The dose-response experiment identifies a dose of a herbicide that provides a 50% reduction in shoot biomass (21).

In the NGR of Australia, there is variability in control of *E. colona* with glyphosate. We hypothesized that the dose required to reduce 50% growth of the plant (GR$_{50}$ value) may vary between biotypes due to development of different levels of glyphosate resistance. It was also hypothesized that the reproduction potential of these biotypes may differ due to variability in the resistance factor. Information on resistant factor, and growth and reproduction behavior of these biotypes is limited in the NGR of Australia. Keeping these points in view, this study was planned to evaluate the growth, reproduction behavior and level of glyphosate resistance in different biotypes of *E. colona*. In this study, one experiment evaluated the growth and reproduction behavior of 10 biotypes of *E. colona* from the NGR of Australia and another experiment evaluated the sensitivity of these biotypes to glyphosate.

Results and discussion

Growth and seed production
Amongst biotypes, the final plant height ranged from 53 to 70 cm, where the lowest was B17/35 and highest was B17/16. Biotypes B17/16, B17/17 and B17/25 attained a similar height, however, they were taller than biotypes B17/34 and B17/35 (Table 1). Tiller number among different biotypes ranged between 30 to 52 plant\(^{-1}\), where the lowest was B17/25 and highest was B17/49 (Table 1). Biotypes B17/7, B17/12, B17/13 and B17/49 produced similar tiller numbers plant\(^{-1}\), however, their tiller production was higher than biotypes B17/25 and B17/35. Leaf numbers in different biotypes varied from 124 to 192 leaves plant\(^{-1}\), where the lowest was biotype B17/16 and highest was biotype B17/34. Leaf production (numbers plant\(^{-1}\)) remained similar for biotypes B17/34, B17/35 and B17/49, however, leaf production in these biotypes was higher than biotypes B17/16 and B17/25. All biotypes produced similar numbers of seed heads except for B17/25, which produced lower numbers than the other biotypes (Table 1).

The weight of seed heads among different biotypes varied from 6.2 to 9.9 g plant\(^{-1}\). It was similar for biotypes B17/7, B17/12, B17/25, B17/34, B17/35, and B17/37 (6.2 to 7.9 g plant\(^{-1}\)), however, these biotypes had a lower seed head weight than biotypes B17/16 (9.8 g plant\(^{-1}\)) and B17/49 (9.9 g plant\(^{-1}\)). Shoot biomass among different biotypes ranged between 20.9 to 27.3 g plant\(^{-1}\) (Table 1). Shoot biomass remained similar for biotypes B17/13, B17/16 and B17/49, however, in these three biotypes, shoot biomass was significantly higher than biotypes B17/34, B17/35 and B17/37. Root biomass did not vary among biotypes (Table 1).

Time taken to seed head initiation in different biotypes varied from 40 to 48 d.

Biotypes B17/7, B17/12, B17/13, and B17/17 took a similar time for seed head initiation (40-42 d) and produced seed heads earlier than biotypes B17/16, B17/25 and B17/35, of which B17/35 took the longest (48 d). Seed production in different biotypes varied from 5380 to 10244 seeds plant\(^{-1}\); where the lowest was biotype B17/25 and highest was biotype B17/13.
Biotypes B17/12, B17/13, B17/34 and B17/49 produced a similar number of seeds (8298-10244 plant$^{-1}$), with their seed production being higher than biotypes B17/25 and B17/35. A linear positive correlation was found for seed number with plant height ($r = 0.67$), tiller number plant$^{-1}$ ($r = 0.89$), leaf number plant$^{-1}$ ($r = 0.73$), seed heads plant$^{-1}$ ($r = 0.78$), seed head weight ($r = 0.79$), shoot biomass ($r = 0.77$) and root biomass ($r = 0.46$) (Table 2). Shoot biomass had a negative relation with days taken to seed head initiation ($r = -0.54$) (Table 2). Plant height, tiller production and seed head weight also had a negative relation with days taken to seed head initiation. Root biomass had a positive correlation with tiller production, leaf production and shoot biomass.

The results of this study demonstrated that characteristics like tall nature and high tillering capacity allow *E. colona* biotypes to produce a high leaf number that resulted in a large number of seed heads and seeds. Therefore, there is a need to target tiller production in *E. colona* to reduce seed numbers. A recent study on crop-weed interference suggested that crop competition could reduce tiller numbers in *E. colona* (22). In Australia, farmers are following wide and skip row spacing in crops such as cotton (*Gossypium hirsutum* L.), mungbean [*Vigna radiata* (L.) R. Wilczek] and sorghum [*Sorghum bicolor* (L.) Moench]; therefore, wide space between the rows could provide a better opportunity to *E. colona* biotypes with a high tillering capacity nature as compared to when crops are sown in narrow rows. In these environments (wide rows and fallows), *E. colona* could attain its high tillering potential and could produce a high seed number. *E. colona* in the present study produced tillers in the range of 39 to 52 plant$^{-1}$; however, in a previous study conducted in Greece, it produced tillers in the range of 115 to 131 plant$^{-1}$ (23). This difference could be due to genotype x environment interactions and differential pot size. In the present study, we observed that biotypes B17/13 and B17/49 had higher tillers than biotypes B17/25 and B17/35. This also suggested that genotypes and environmental interactions played a role in
influencing tiller numbers per plant in *E. colona* biotypes. The regions where biotypes are of high tillering capacity are expected to suffer a high crop yield loss due to high *E. colona* competition.

The high seed number observed in biotypes B17/12, B17/13, B17/34 and B17/49 was largely attributed to a high number of tillers, leaves, and seed heads. Our study also revealed that seed head weight also influences seed number. The number of leaves and seed heads were similar between biotypes B17/13 and B17/35; however, seed production was lower in B17/35, which could be due to the lower tiller production and seed head weight in B17/35. The time taken to seed head initiation in the present study was similar to a study conducted in northern Greece, in which *E. colona* attained seed heads between 39 to 45 days after transplanting (23). The biotype B17/35 (selected from the Moree region) took a longer time for seed head initiation than other biotypes (Figure 1). In a previous study in South-East Asia, 12 *E. colona* biotypes were studied and it was found that time for seed heads in different biotypes varied with latitude and plants from a high latitude attained seed heads earlier than from a low latitude (24). This suggested that growth duration in different biotypes of *E. colona* may vary with geographical location. In the present study, the negative relationship between seed head initiation and seed number revealed that late-maturing biotypes produced fewer seeds as was the case for biotypes B17/16 and B17/35 when compared with biotype B17/13. Time taken to seed head initiation also had a negative relationship with plant height, tiller number, and shoot biomass. These results suggest that diversity in *E. colona* traits could result in differential responses to herbicides, cultural practices, and resistance evolution. For example, the early vigor trait in *E. colona* is an important trait that could affect early crop-weed competition (25) and therefore, management of such biotypes at an early stage is required to increase crop production and reduce the weed seed bank in the soil.
In this study, *E. colona* biotypes differed in their seed production potential, which ranged between 5380 to 10240 seeds plant\(^{-1}\). Differential seed production in *E. colona* biotypes could play an effective role in its spread and population establishment (26). High seed yields in biotypes B17/12, B17/13, B17/34 and B17/49 were largely based on a greater number of seed heads and leaf numbers plant\(^{-1}\). High production of leaves in these biotypes probably maintained a better supply rate of carbon assimilates to seeds. In one study on *Brassica*, it was found that variation in the supply of carbon assimilates to seeds at or immediately after anthesis could cause a variation in seed production in different biotypes (27). Some authors also highlighted the role of the supply of carbon assimilates in determining the seed number in pea (*Pisum sativum* L.) plant (28).

The present study also revealed that tiller number per plant played a large role in seed production along with leaf number per plant. Biotype B17/35 had high leaf production but could not produce higher amounts of seeds like B17/13 and B17/34 did, because it had lower tiller production than B17/13 and B17/34. Although this study revealed that the supply of carbon assimilates after anthesis could be a major factor in determining seed production, we could not rule out the possibility of hormonal factors for variation in seed production in these biotypes. These results suggest that there is also a need to study nutritional and hormonal factors for variation in seed production in these biotypes (27). Our study (second experiment) also found that the GR\(_{50}\) value of glyphosate for these biotypes varied. These results suggest that in these biotypes, seed viability, seed persistence and fitness penalty may differ and therefore systematic studies need to be investigated. Such knowledge of seed production in these biotypes is required for understanding the evolution and spread of herbicide resistance particularly for herbicide-resistant biotypes.
Response to glyphosate

Out of 10 *E. colona* biotypes collected from the NGR of Australia, three biotypes (B17/16, B17/34 and B17/35) had greater than 80% survival following treatments with 325 to 2600 g a.e. ha\(^{-1}\) glyphosate. The probit analysis details for each biotype along with their level of significance is presented in Table 3. The dose-response study of glyphosate for these biotypes showed a wide range of responses (Figure 2). The GR\(_{50}\) value of the tested biotypes ranged from 217 to 2159 g ha\(^{-1}\) (Figure 2). The susceptible biotype B17/37 was easily controlled with glyphosate and had a GR\(_{50}\) of 217 g ha\(^{-1}\), below the normal use rate of this herbicide (650 g ha\(^{-1}\)). The GR\(_{50}\) values of biotypes B17/16, B17/34 and B17/35 were 719, 2159 and 884 g ha\(^{-1}\), respectively, making them 3, 10 and 4-fold resistant to glyphosate compared with the susceptible biotype B17/37. The most resistant biotype B17/34 was from the Goondiwindi region, whereas the next most resistant biotypes, B17/35 and B17/16, were from the Moree and Narrabri regions, respectively. This study has revealed that *E. colona* biotypes in the NGR of Australia have different levels of resistance to glyphosate. No-till farming is quite popular in the NGR of Australia for moisture conservation. Therefore, growers use glyphosate in summer fallows to kill weeds and conserve moisture. Repeated and intensive use of glyphosate in this region has evolved glyphosate resistance in these biotypes (11). Risk of glyphosate resistance evolution for weeds is higher in those areas where glyphosate has been used for a long time and with minimal soil disturbance (29). In Australia, glyphosate-tolerant cotton varieties are very popular among cotton growers. Glyphosate-resistant *E. colona* biotypes may create serious situations in that production environment. The resistant factor for glyphosate in this study was similar to the first reported case of glyphosate resistance in Australia (7 to 11-fold resistance compared with a susceptible population); but that study was reported for rigid ryegrass (*Lolium rigidum* Gaud.) (30).
Earlier, glyphosate resistance in *E. colona* biotypes was also reported in Australia (11, 31). There are a number of mechanisms responsible for glyphosate resistance (32), and different mechanisms may result in a different level of resistance (33). Therefore, these studies suggest that these resistant biotypes may not carry the same resistance allele, which needs to be investigated. Many reports of glyphosate resistance in different weeds highlight that the reliance on glyphosate for weed control, in the long run, exerts a substantial selection pressure on weeds (34,35,36,37,38,39,40). Therefore, integrated weed control should be strengthened to reduce selection pressure on these resistant biotypes, particularly in cotton paddocks. It is quite possible that the mechanism of glyphosate resistance in Australian *E. colona* biotypes might be different from resistant *E. colona* biotypes reported from California (41) as Australian biotypes of *E. colona* have adapted to a dry environment. Therefore, a systematic study is required to understand the evolution of glyphosate resistance in these biotypes. The evolution of glyphosate resistance in tropical *E. colona* in Australia suggests that there is a large risk with increased use of glyphosate in fallows and improved stewardship guidelines for glyphosate use are required in the NGR of Australia.

The present study on *E. colona* biotypes has increased our understanding of the physiological basis of differences in seed production due to variations in morphological characteristics and resistance behavior. It highlighted that growth parameters such as high tiller production in *E. colona* biotypes leads to more leaves and in turn high seed production. The study further demonstrated that growth behavior and seed production potential in these biotypes had no correlation with the resistance index. However, this research has posed more questions than it has answered. This study suggested that biotypes such as B17/34 that are highly glyphosate-resistant, and also produced a high seed number (9300 seeds plant\(^{-1}\)) are very problematic. Therefore, systematic research on weed biology, physiology and resistance mechanism is required to answer these questions for better understanding. This study also
suggested that there is a need to understand the likelihood of resistance transfer from resistant
to susceptible biotypes through pollen-mediated gene flow and introgression. Such
knowledge could be useful in restricting the further spread of glyphosate-resistant biotypes of
*E. colona*.

**Materials and methods**

The study was conducted at the QAAFI weed science laboratory and screen house of the
University of Queensland, Gatton, Australia. Seeds of 10 different biotypes of *E. colona* were
collected from the NGR of Australia in March 2017. The coordinates of these biotypes are
depicted in Figure 1. The seeds of each biotype were cleaned and stored in shade.

**Growth response experiment**

In this experiment, 10 biotypes of *E. colona* were grown in pots replicated four times. The
pots were kept on benches placed outside the screen house. Pots were filled with potting mix
(Crasti & Company Pty Ltd, Sydney, Australia). Initially, 10 seeds were sown per pot at 1 cm
depth and after establishment, one plant per pot was maintained. The experiment was
consducted twice. The first run was started on 27 September 2018 and harvested on 6
December 2018. The second run was started on 3 December and harvested on 5 February
2019. Pots used in the experiment were 20 cm in height and arranged in a completely
randomized design. The pots were regularly irrigated.

At maturity, plant height was measured from the base of the plant to the tip of the
uppermost leaf of the plant. Days taken to seed head initiation was recorded in each pot. For
estimating seed production per head, two intact seed heads were chosen randomly from each
plant. For the total number of seeds, each rachilla segment (pedicel base) was counted and
then, averaged for seeds per head. At harvesting time, tiller numbers, leaf numbers and seed
heads per plant were also counted. Harvesting was done when ~80% seed heads matured.
At harvest, seed heads were separated from the plants for measuring shoot biomass. After that, all aboveground shoot biomass from each plant was placed separately in a paper bag and dried in an oven at 70 °C for 72 hours before being weighed. For root weight data, pots containing potting mix with the root system were first dried in an oven at 70 °C for 72 hours. After that, roots were removed from each pot by shaking. Root biomass of each plant was then measured. Drying of potting mix in an oven helped in the separation of the root system from the potting mix.

**Glyphosate dose-response experiment**

Seeds of 10 biotypes were sown in pots (9 cm diameter and 10 cm height) filled with potting mix (Crasti & Company Pty Ltd, Sydney, Australia). Initially, 10 seeds were sown per pot at 1 cm depth and after establishment, five plants per pot were maintained. Pots were kept in a screen house under natural light and temperature conditions. The experimental design was a factorial with four replicates where the first factor was biotype and the second factor was glyphosate dose [0x (no herbicide; control), 0.5x, 1x, 2x, and 4x]. The 1x dose was the recommended dose (650 g a.e. ha⁻¹) for glyphosate. The experiment was conducted twice. The first run was started on 5 December 2018 and harvested on 14 January 2018. The second run was started on 25 January 2019 and harvested on 6 March 2019. Glyphosate application was done on 24 December 2018 in the first run and 13 February 2019 in the second run. Plants were kept well-watered and fertilized.

Glyphosate was sprayed using a research track sprayer. Plants were treated at the 4-5 leaf stage using a spray volume of 108 L ha⁻¹ and Teejet XR 110015 flat fan nozzles were used. Plants were allowed to grow for 21 days after treatment (DAT) to determine glyphosate sensitivity. Plant survival was assessed 21 DAT, and plant aboveground biomass was harvested, dried for 72 hours at 70 °C, and weighed.
Statistical analyses

The first experiment was conducted in a completely randomized design and the second experiment was conducted in a completely randomized design with a factorial arrangement. In both experiments, there was no interaction between experimental runs and treatments; therefore, the data of the two runs were pooled for ANOVA. All the data met assumptions of normality of residuals and homogeneity of variance. Data of the first experiment were subjected to analysis of variance (ANOVA) using the software Elementary Designs Application 1.0 Beta (AgriStudy. com: www.agristudy.com) (verified with GENSTAT 16th Edition; VSN International, Hemel Hempstead, UK). Treatment means were separated using Fisher’s protected LSD at P≤0.05.

For the second experiment, GR$_{50}$ (the dose required to kill 50% of the growth of plants) estimates were generated using Probit analysis [IBM SPSS Statistics 20.0 (SPSS, Inc., Chicago, IL, USA)]. The level of significance was tested with a Chi-Square goodness of fit test. When the calculated value of Chi-Square goodness of fit test was greater than the table value, the null hypothesis was rejected and it was concluded that there was a significant difference between the observed and the expected value and vice versa with values lower than the table value. The resistance index (resistance/susceptibility ratio) was calculated on the basis of the GR$_{50}$ value to compare the resistance level among different biotypes.

Author Contributions

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Data Availability Statement

All relevant data are within the paper and its Supporting Information files.

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Conflicts of Interest

The authors declare no conflicts of interest.

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activity in glyphosate-resistant junglerice (*Echinochloa colona*) from California. Pest Management Science, 2015. 71: p. 996-1007.
Table 1. Morphological traits and seed production potential of different biotypes of *Echinochloa colona*

| Biotype | Plant height (cm) | Tiller (number plant\(^{-1}\)) | Leaf (number plant\(^{-1}\)) | Seed head (number plant\(^{-1}\)) | Seed head weight (g plant\(^{-1}\)) | Shoot biomass (g plant\(^{-1}\)) | Root biomass (g plant\(^{-1}\)) | Days to seed head initiation (d) | Seed production (number plant\(^{-1}\)) |
|---------|-------------------|-------------------------------|-----------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------------|-----------------------------------|
| B17/7   | 62.9              | 47                            | 134.7                       | 52.7                          | 7.7                             | 24.8                          | 12.7                          | 42                             | 7022                              |
| B17/12  | 61.2              | 47                            | 163.6                       | 63.9                          | 7.5                             | 24.2                          | 13.0                          | 41                             | 8837                              |
| B17/13  | 63.7              | 50                            | 156.3                       | 59.4                          | 8.5                             | 25.1                          | 18.0                          | 40                             | 10244                             |
| B17/16  | 70.3              | 40                            | 124.1                       | 53.5                          | 9.8                             | 27.3                          | 10.1                          | 45                             | 6986                              |
| B17/17  | 66.9              | 41                            | 134.5                       | 60.5                          | 8.1                             | 22.7                          | 9.7                           | 42                             | 7801                              |
| B17/25  | 66.7              | 30                            | 132.1                       | 36.6                          | 6.2                             | 22.1                          | 13.7                          | 45                             | 5380                              |
| B17/34  | 56.2              | 43                            | 192.1                       | 62.9                          | 7.9                             | 20.9                          | 12.5                          | 44                             | 9295                              |
| B17/35  | 53.1              | 39                            | 185.9                       | 65.1                          | 6.2                             | 20.9                          | 16.1                          | 48                             | 6130                              |
| B17/37  | 63.2              | 43                            | 162.1                       | 59.0                          | 7.5                             | 21.1                          | 12.4                          | 43                             | 6387                              |
| B17/49  | 62.9              | 52                            | 165.7                       | 63.7                          | 9.9                             | 25.4                          | 11.9                          | 44                             | 8298                              |
| LSD     | 5.7               | 7.0                           | 30.5                        | 14.2                          | 1.7                             | 3.8                           | NS                           | 2.7                            | 2136                              |

NS: nonsignificant
| Parameter                        | Plant height (cm) | Tiller (number plant\(^{-1}\)) | Leaf (number plant\(^{-1}\)) | Seed head (number plant\(^{-1}\)) | Seed head weight (g) | Shoot biomass (g plant\(^{-1}\)) | Root biomass (g plant\(^{-1}\)) | Days to seed head initiation (d) | Seed production (number plant\(^{-1}\)) |
|---------------------------------|-------------------|--------------------------------|-----------------------------|-----------------------------------|---------------------|-------------------------------|----------------------------|---------------------------------|---------------------------------------|
| Tiller (number plant\(^{-1}\))  | 1                 |                                |                             |                                   |                     |                               |                             |                                 |                                       |
| Leaf (number plant\(^{-1}\))    | 0.77*             |                                |                             |                                   |                     |                               |                             |                                 |                                       |
| Seed head (number plant\(^{-1}\))| 0.55*             | 0.77*                         |                             |                                   |                     |                               |                             |                                 |                                       |
| Seed head weight (g plant\(^{-1}\)) | 0.59*            | 0.87*                         | 0.87*                       |                                   |                     |                               |                             |                                 |                                       |
| Seed head weight (g plant\(^{-1}\)) | 0.82*            | 0.84*                         | 0.51*                       | 0.68*                            |                     |                               |                             |                                 |                                       |
| Shoot biomass (g plant\(^{-1}\)) | 0.96*            | 0.88*                         | 0.70*                       | 0.72*                            | 0.83*               |                               |                             |                                 |                                       |
| Root biomass (g plant\(^{-1}\)) | 0.36              | 0.47*                         | 0.52*                       | 0.39                             | 0.16                | 0.47*                         |                             |                                 |                                       |
| Days to seed head initiation (d) | -0.54*            | -0.65*                        | -0.26                       | -0.42                            | -0.55*              | -0.54*                        | -0.16                       |                                 |                                       |
| Seed production (number plant\(^{-1}\)) | 0.66*            | 0.88*                         | 0.70*                       | 0.78*                            | 0.73*               | 0.78*                         | 0.53*                       | -0.68*                          | 1                                    |

Critical value of \( r \) at 5% = 0.44; * indicates significant relation
Table 3. Probit analysis detail [Intercept $a + bx$ (covariate $x$ are transformed using the base 10.0 logarithm)] for different *Echinochloa colona* biotypes

| Biotype   | $a$ Intercept | $b$ Estimate | Pearson Goodness-of-fit | Significance level (Chi square) |
|-----------|---------------|--------------|-------------------------|--------------------------------|
| B17/7     | -9.23         | 3.95         | 0.95                    | 0.62                           |
| B17/12    | -9.37         | 3.94         | 3.98                    | 0.14                           |
| B17/13    | -9.25         | 3.63         | 2.42                    | 0.30                           |
| B17/16    | -1.55         | 0.54         | 0.78                    | 0.68                           |
| B17/17    | -7.76         | 3.31         | 1.10                    | 0.58                           |
| B17/25    | -13.8         | 5.51         | 0.01                    | 0.99                           |
| B17/34    | -5.25         | 1.57         | 5.68                    | 0.06                           |
| B17/35    | -2.94         | 0.99         | 5.48                    | 0.06                           |
| B17/37    | -3.11         | 1.33         | 3.19                    | 0.20                           |
| B17/49    | -4.41         | 1.66         | 3.25                    | 0.20                           |
Figure 1. Location of 10 biotypes of *Echinochloa colona* selected from the northern grain region of Australia
Figure 2: a) GR$_{50}$ dose of glyphosate and b) glyphosate-resistant/susceptible factor in different biotypes of *Echinochloa colona*.