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MORPHOLOGICAL AND REPRODUCTIVE TRAIT VARIATION OF ELEUSINE INDICA ACROSS HABITATS WITH CONTRASTING DISTURBANCE REGIMES

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ABSTRACT

Eleusine indica (L.) Gaertn., commonly known as goosegrass, is a widely distributed weed species in the Poaceae family. Its success as an agricultural weed is associated with its prolific seed production, strong competitiveness with cultivated crops, extended germination period, and ability to thrive under a wide range of environmental conditions. This study investigated reproductive and genetic variation among *E. indica* populations collected from different habitats. A total of 45 mature plants were sampled from three habitat types and evaluated based on seven reproductive morphological characteristics. Genetic diversity was examined using the psbA–trnH intergenic spacer (IGS) region, with 18 representative samples selected for sequencing. Significant differences ($p < 0.05$) were observed among habitats for spike length, peduncle length, and seed length, suggesting the influence of environmental conditions and adaptive responses. Roadside populations exhibited shorter spike and peduncle lengths, whereas farm and wasteland populations produced relatively larger reproductive structures, indicating morphological responses to habitat disturbance intensities. Analysis of the psbA–trnH sequences revealed low genetic variation, with only two haplotypes identified among the samples. Similarly, limited polymorphism was detected among *Eleusine* species, including *E. indica*, *E. multiflora*, and *E. coracana*, indicating a high degree of genetic similarity and conservation within the genus. The findings indicate low reproductive variation and low genetic diversity of *E. indica* across the three habitats, as revealed by the psbA–trnH IGS marker, might be due to the close genetic relationship among populations and the limited resolution of this chloroplast marker in detecting intraspecific variation.

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INTRODUCTION

Weeds represent strong models for studying rapid evolutionary responses under agricultural pressure and changing environmental conditions. *Eleusine indica* (L.) Gaertn., commonly known as goosegrass, is a widespread member of the Poaceae family and is recognized as both an ecologically aggressive and economically important weed species. It has been reported as a major weed in more than 60 countries and across over 46 crop species, where it significantly reduces productivity in crops such as cereals, legumes, cotton, tobacco, and vegetables (Ma *et al.*, 2019; Adoho *et al.*, 2021). Its growth form is highly plastic and influenced by vegetation density, and it can reach up to approximately 40 cm in height under favourable conditions. As a monoecious, wind-pollinated species, *E. indica* has a high capacity

for reproduction and dispersal, which facilitates its successful establishment in diverse agricultural and disturbed habitats (Kurniadie *et al.*, 2023. Although its exact centre of origin remains uncertain, it is generally believed to have originated in tropical and temperate regions of Asia and Africa. From these regions, it has spread widely and is now commonly found throughout tropical and subtropical zones, particularly across Africa, Asia, and South America (Ma *et al.*, 2019). Its ecological success is largely attributed to its adaptability, competitive ability, and tolerance to a wide range of environmental conditions. Increasing reports of herbicide resistance in *E. indica* further highlight its adaptive capacity under intensive agricultural management. Such success is also strongly associated with high phenotypic plasticity, enabling adjustment of growth and reproductive strategies across varying environmental conditions (Plaza *et al*., 2021).

E. indica succeeds ecologically because of its strong physiology and ability to colonize bare or damaged areas quickly (Patel *et al.*, 2023). Because of its wide variety in plant shape and seed development, the species can survive in cultivated areas, near roads and in disturbed lands (Kurniadie *et al.*, 2023; Patel *et al.*, 2023). It is often present on vegetable farms, in orchards, oil palm and rubber plantations, as well as in wastelands and along roadsides in Malaysia. Its ability to alter is closely linked to the C_4 route of photosynthesis which helps it use water wisely and tolerate droughts, systems that are useful in changing and degraded environments.

An *E. indica* plant can produce as many as 140,000 seeds and these seeds travel and settle quickly, making the plant durable in several different types of environments (Ma *et al*., 2019). The weed demonstrates longer germination times and a lot of seedling emergence within short period of time helps it to become an aggressive weed species. Although seed size, shape, surface texture and color serve as an indicator for reproductive success, but how they function in this process remains unknown (Hani *et al.*, 2017). Within populations, variation in physical traits often surpasses that between different species and these differences can influence the environment. It is very important to understand phenotypic plasticity when designing weed control methods. While *E. indica* is significant around the world, little research has been done on how it varies morphologically and genetically in Malaysia.

Genomic studies offer powerful tools for revealing evolutionary patterns and adaptive traits in weed populations. While nuclear and mitochondrial genomes are complex and large, chloroplast genomes, especially the intergenic spacer regions are comparatively conserved and easier to analyze (Henry, 2022). Among them, the *psbA-trnH* intergenic spacer (IGS) has shown promise as a phylogenetic marker due to its high amplification success and informative nucleotide variation (Degtjareva *et al*., 2012). In addition, the relatively high sequence variability of the psbA-trnH region makes it useful for detecting genetic variation among closely related populations and species, supporting its application in intraspecific diversity studies. This region lies between the psbA gene and the histidine tRNA (trnH) gene and plays a role in gene expression regulation. Although this marker has been used effectively in genera such as *Rhododendron*, *Dendrobium*, and *Compsoneura*, it has not yet been applied to *E. indica*, leaving a gap in our understanding of its genetic diversity.

Given these knowledge gaps, this study was designed to evaluate both the reproductive morphological variation and genetic diversity of *E. indica* across three

distinct habitats. The study hypothesizes that *E. indica* populations may display significant genetic and morphological divergence in response to varying environmental conditions. The specific objectives are: (1) to evaluate reproductive and vegetative morphology variations and distinctness of *E. indica* in three different habitats, and (2) to assess genetic variation using the *psbA-trnH* intergenic spacer marker. Understanding the morphological and genetic variation of E. indica may provide new information into its persistence and adaptive potential, which will contribute to the development of sustainable weed management practices.

MATERIALS AND METHODS

Study Area

Field sampling was carried out in Kampar, Perak across sites representing different levels of human disturbance. Three habitat types were selected: conventional farms (F) as high disturbance, roadsides (R) as moderate disturbance, and wastelands (W) as low disturbance (Figure 1). For each habitat type, three locations were chosen and five mature *E. indica* plants were collected per location, giving a total of 45 samples. GPS coordinates were recorded for all sampling sites. Disturbance intensity was classified based on land-use practices following Bommarco *et al*. (2010) and Hantsch *et al*. (2013). Farms were associated with frequent tillage and agrochemical inputs, roadsides with periodic maintenance and vegetation clearing, and wastelands with minimal human management and disturbance.

Figure 1. The conventionally managed farm (F) (left), roadside (R) (middle) and wasteland habitat (W) (right).

Plant Sample Collection and Reproductive-Vegetative Morphological Characterization

A total of 45 mature goosegrass plants with visible seedheads were collected in a mosaic pattern within each site to reduce potential sampling bias caused by local gene flow or ecological clustering (Bommarco *et al.*, 2010). Whole plants were collected and cleaned of soil and debris prior to morphological analysis (Hassan *et al.*, 2020). Each plant sample was assessed for the reproductive and vegetative morphological traits. The traits assessed were total number of seedheads per plant, number of spikes per seedhead, spike length, peduncle length, seed weight, seed length, and seed width. Vegetative morphological traits were also recorded, including plant height, internode length, flag leaf width, and number of tillers per plant. Seeds were manually separated from plant debris by hand rubbing. Measurements of seed length and width were conducted using the Motic Camera Plus 2.0 imaging system for accuracy.

Plant Morphological Data Analysis

Data were analyzed using SAS OnDemand for Academics. A nested Analysis of Variance (ANOVA) was performed to detect statistically significant differences ($p < 0.05$) in reproductive traits across habitat types. Duncan's Multiple Range Test (DMRT) was used post hoc to differentiate between means. Additionally, boxplots were generated to visualize trait variability. Reproductive trait data were classified into four quartile-based categories and used to generate a distance matrix with GenAlEx 6.51b2.

Genetic Diversity Assessment

DNA Extraction

Young leaf tissues (0.03–0.05 g) were collected from each of the 45 mature *Eleusine indica* plants sampled from the nine study locations in Kampar, Perak, and preserved in silica gel in sterile biohazard specimen bags. Silica gel was periodically replaced to maintain desiccation prior to DNA extraction. DNA was extracted using the Biospin Plant DNA Extraction Kit Cat#BSC13S1 following the manufacturer's protocol. Plant tissue samples (about 0.03 g to 0.05 g) were combined with 900 µL LP buffer in a mortar and the mixture were grinded together using pestles to release the DNA within the samples. Then, the tissue solution was transferred to a 1.5 mL microcentrifuge tube and incubated at 65°C for 15 minutes. A volume of 150 µL DA buffer was added to the lysate and mixed thoroughly using vortex machine. The lysate is then centrifuged for 5 minutes at 12,000 rcf. The supernatant was transferred to a new 1.5 mL tube and mixed with 1.5 volumes of the P buffer to make the DNA bound to Biospin membrane on the next step onwards. Then, the mixture was transferred to a spin column and centrifuged at 12,000 rcf for 1 minute. After that, the flow-through is being discarded. Another volume of 500 µL of G Binding Buffer added into spin column and centrifuged again at same speed for 30 seconds. After discard the flow through from previous step, 600 µL Washing buffer was added to the spin column and centrifuged at 12,000 rcf for 30 seconds. The flow through discarded again. Step 8 was repeated one more time to wash thoroughly denatured protein and other contaminants. An additional 1 minute of centrifugation at 12,000 rcf again before transfer the spin column to a new 1.5 mL tube. A 100 µL of Elution buffer was added to the tube and incubated at room temperature for 2 minutes to unbound the DNA from membrane. Lastly, another centrifugation at 12,000 rcf for 2 minutes. The buffer in the microcentrifuge tube contained the DNA. Steps included cell lysis, protein precipitation, DNA binding, washing, and elution. Extracted DNA was stored at −20 °C. DNA purity and concentration were determined spectrophotometrically using the A260/A280 ratio.

PCR Amplification, Gel Electrophoresis, Purification, and DNA Sequencing

The chloroplast intergenic spacer region psbA–trnH, a highly variable non-coding marker, was selected for genetic diversity analysis (Dwiati *et al.,* 2023). PCR amplification was performed using universal primers, with the forward primer 5'-CGCGCATGGATTCACAAT-3' and the reverse primer 5'-GTTATGCATGAACGTAAT-3'. Each 50 µL PCR reaction consisted of 25 µL of 2× PCR Master Mix (final concentration 1×), 2 µL of forward primer (0.4 µM), 2 µL

of reverse primer (0.4 µM), 6 µL of DNA template, and 15 µL of distilled water. Amplification was carried out in a single-block thermal cycler under the following cycling conditions: an initial denaturation at 94 °C for 2 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s; with a final extension at 72 °C for 5 min and a hold at 10 °C indefinitely (Kumar *et al*., 2016; Duan *et al.*, 2019). PCR products (5 µL) were then resolved on a 1.2% agarose gel prepared in 1× TAE buffer, electrophoresed at 300 V for 45 min, and visualized under UV illumination using a 1 kb DNA ladder for fragment size confirmation.

PCR products were subsequently purified using the FavorPrep GEL/PCR Purification Mini Kit (Cat# FAGCK 001) following the manufacturer's protocol, which included DNA binding, washing, and elution in 40 µL of elution buffer, with final elution performed by centrifugation at 18,000 rcf. Due to sequencing cost limitations, 18 representative PCR products were selected for Sanger sequencing, ensuring representation across all habitat types and sampling locations to capture genetic variation within the study area. The resulting sequences were edited and trimmed using BioEdit v7.2, and aligned using ClustalX2. Polymorphic sites were manually inspected to identify sequence variation among samples

RESULTS AND DISCUSSION

Reproductive and Morphological Variation Across Habitats

Reproductive trait analysis of *E. indica* from farm, roadside, and wasteland habitats revealed significant variation in certain morphological characteristics (Table 1). Among the seven traits measured, spike length, peduncle length, and seed length were found to be significantly different between habitats. Conversely, number of seedheads per plant, number of spikes per seedhead, seed weight and seed width were not significantly different across habitats ($p > 0.05$). Vegetative morphological traits of *E. indica* also showed clear variation across habitats (Table 2). Plant height, internode length, and flag leaf width were significantly higher in farm and wasteland populations compared to roadside populations, while tiller number did not differ significantly among habitats. Farm and wasteland plants exhibited greater overall vegetative growth, suggesting more favourable growth conditions with better resource availability. In contrast, roadside populations showed reduced plant height and internode length, likely due to environmental stress such as soil compaction, limited moisture availability, and exposure to pollutants. The observed differences in vegetative morphology may influence reproductive performance, as plant morphology is closely linked to assimilate allocation and inflorescence development.

Table 1. The reproductive morphology of *E. indica* in three different habitats

Habitats	Number of Seedhead/ Plant	Number of Spikes/ Seedhead	Spikes Length (cm)/ Seedhead	Peduncle Length (cm)/ Plant	Seed Weight (g) (100 seeds)	Seed Length (mm)	Seed Width (mm)
Farm	4.47±2.97[a]	5.13±1.95[a]	7.81±1.64[a]	20.09±6.12[a]	0.02±0.01[a]	1.14±0.13[a]	0.58±0.08[a]
Roadside	5.87±4.06[a]	4.33±1.50[a]	5.64±1.46[b]	12.32±3.30[b]	0.03±0.01[a]	1.29±0.10[b]	0.64±0.07[a]
Wasteland	6.27±2.28[a]	4.20±0.97[a]	7.32±2.00[a]	15.92±6.02[b]	0.03±0.01[a]	1.22±0.14[ab]	0.64±0.08[a]

Table 2. The vegetative morphology of *E. indica* in three different habitats

Habitat types	Plant height (cm)	Internode length (cm)	Flag leaf width (mm)	Number of tillers
Farm	57.29±16.08[a]	7.81±3.14[a]	5.81±0.94[a]	14.00±6.56[a]
Roadside	39.39±8.68[b]	4.06±1.85[b]	4.68±0.70[b]	13.20±12.13[a]
Wasteland	53.19±14.66[a]	7.64±1.85[a]	5.38±0.92[a]	15.86±6.65[a]

Plants in the farm area had from four to six seedheads each and wasteland plants had the most at an average of about six seedheads. The similarity of the results ($p > 0.05$) across the groups points to a stable reproductive feature for this population. Kerr *et al*. (2019) also observed that *E. indica* plants growing under well-maintained conditions, such as in turfgrass areas, produced fewer seedheads. The use of plant growth inhibitors in crop cultivation is believed to suppress reproductive development by altering hormone levels, particularly by reducing gibberellin production. Besides, under competition with crop plants for limited resources, *E. indica* tends to allocate more biomass toward vegetative growth (leaves and stems) rather than reproductive structures such as seeds, reflecting a common plastic response in plants under competitive stress (Savić *et al.*, 2025). On the other hand, in places like wastelands with little competition, the plant may produce more seedheads. Variation in seedhead production was highest in roadside populations, likely due to the heterogeneous nature of roadside environments, where soil compaction, pollutant exposure, light availability, and moisture levels vary considerably over short distances. Such environmental inconsistency can impose uneven stress on plant development. Under fluctuating conditions such as drought, flooding events, or elevated temperatures, floral development may become unstable, resulting in greater variability in reproductive output (Asaduzzaman *et al.*, 2022).

There was no significant difference in the number of spikes per seedhead with all populations falling within the normal range of three to seven spikes for *E. indica* (Hooda and Chauhan, 2023). This consistency may be influenced by gene flow between wild and managed areas facilitated by human movement and vehicle activity (Patel *et al.*, 2023), and is also consistent with the species' globally adaptive reproductive strategy that maintains stable floral structures across diverse environments. The slightly higher spike number observed in farm populations may be associated with nitrogen-rich fertilizer application, which enhances inflorescence development during the reproductive phase (Anas *et al.*, 2020; Tan *et al.*, 2020). In

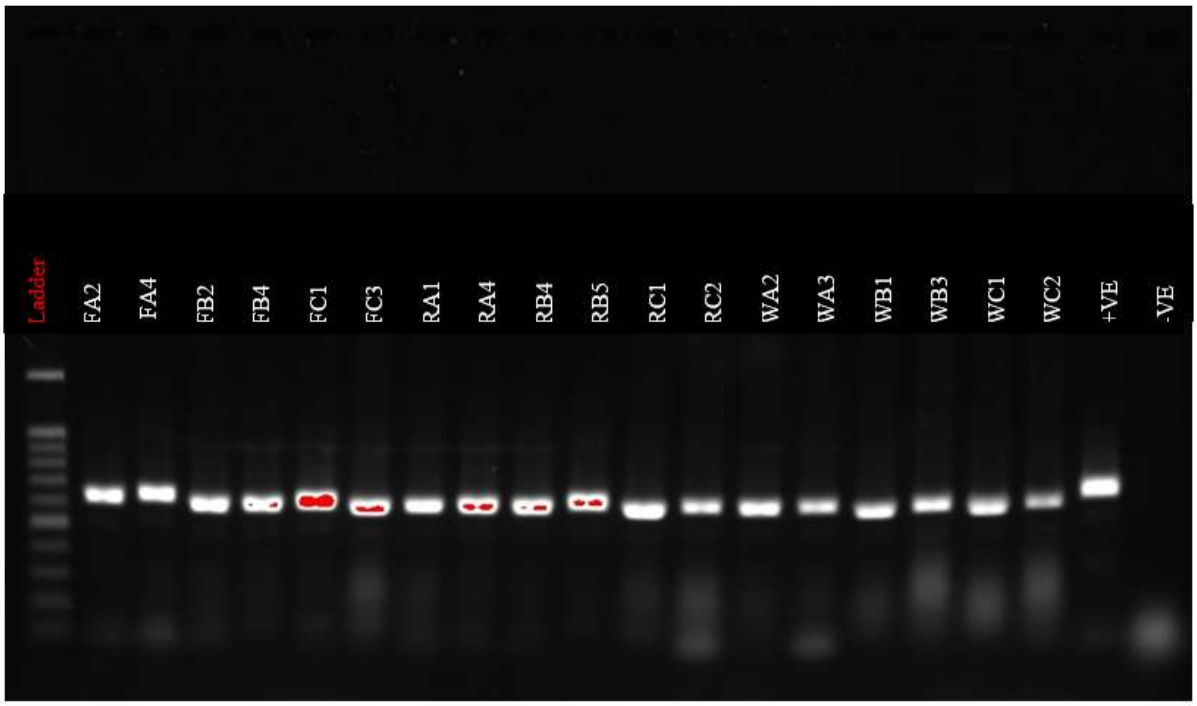
contrast, spike length showed significant variation among habitats ($p < 0.05$), with longer spikes recorded in farmland and wasteland compared to roadside populations. This pattern aligns with Ikhajiagbe *et al.* (2022), who reported reduced spike length in roadside environments due to pollutant stress. Such stress, including exposure to nitrogen oxides, heavy metals, and soil compaction, can impair nutrient uptake by restricting access to essential elements such as calcium, magnesium, and iron, ultimately affecting organ development (Patel *et al.*, 2023). Additionally, limited root space and reduced water availability in compacted roadside soils further constrain growth. Previous studies have also shown that tillering patterns and spikelet structure are closely linked to spike length regulation, both of which may be negatively affected under environmental stress (Guo *et al.*, 2018).

There were significant differences in peduncle length among habitat types, with the longest values recorded in farm populations (20.09 cm) and the shortest in roadside areas (12.32 cm). Longer peduncles are generally associated with taller plants and larger inflorescences, which develop optimally under favourable growth conditions (Khalid *et al.*, 2023). Fertile farm soils with better management practices likely support enhanced growth, particularly where gibberellin availability promotes stem and inflorescence elongation (Buthelezi *et al.*, 2023). In contrast, reduced peduncle length in roadside populations may reflect adaptive responses to water limitation and drought stress, where reduced turgor pressure restricts cell expansion (Khalid *et al.*, 2023). Overall, the variation in peduncle length across habitats indicates strong environmental sensitivity and phenotypic plasticity in *E. indica*. In contrast, seed weight and seed width showed no significant differences among populations across all habitats. This stability suggests a relatively conserved genetic control over seed provisioning, likely maintained despite environmental variation. Such uniformity may also be supported by effective dispersal mechanisms, where seeds are transported via wind, water, or human activity, contributing to gene flow among populations. Additionally, the production of small and lightweight seeds in large quantities enhances dispersal efficiency and establishment success in diverse environments, supporting the invasive potential of the species (Hani *et al.*, 2017).

Seed length showed significant variation among habitats with the longest seeds recorded in roadside populations, followed by wasteland and farm populations. This variation is likely linked to differences in microclimatic conditions such as temperature, moisture, and light, which influence seed development (Chuah *et al.*, 2004). Longer seeds may improve establishment under stressed conditions by enhancing early growth and soil penetration. In nutrient-poor roadside habitats, changes in seed traits may reflect a compensatory response to improve establishment success (John *et al.*, 2012). Vegetative traits showed a similar pattern (Table 2), where plant height, internode length, and flag leaf width were higher in farm and wasteland populations than roadside populations, while tiller number was not significantly different among habitats. This suggests better resource availability in farm and wasteland habitats, whereas roadside conditions such as soil compaction, low moisture, and pollution restrict plant growth. Overall, reproductive traits were relatively stable, while both reproductive structures and vegetative traits showed habitat-related variation. This balance between stability and plasticity supports the ability of *E. indica* to persist across disturbed environments.

Genetic Diversity Analysis using *psbA-trnH* Marker

Genomic DNA was extracted from each of the 18 *E. indica* samples found in the conventional farm, roadside, and wasteland habitat types. The DNA concentrations measured from 264.6 to 3,757.5 ng/µL and were pure with purity values between 1.92 and 2.09 at a 260/280 nm ratio, demonstrating suitable DNA for grey scale sequencing. DNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) based on absorbance measurements at 260 and 280 nm. A single, prominent band of approximately 600–650 bp was produced for *psbA–trnH* intergenic spacer PCR which showed successful amplification (Figure 2). Afterwards, the amplicons were sequenced and the edited and trimmed sequences turned out to be 542 bp (Figure 3).



ladder = 1 kb ladder

Figure 2. Agarose gel electrophoresis of *psb*A–*trn*H PCR products amplified from 18 *Eleusine indica* samples. Lane M: 1 kb DNA ladder; Lane PC: positive control; Lane NC: negative control; Lanes 1–18: *E. indica* samples collected from farm (F), roadside (R), and wasteland (W) habitats. A single amplicon of approximately 600–650 bp was obtained in all samples, while no amplification was observed in the negative control.

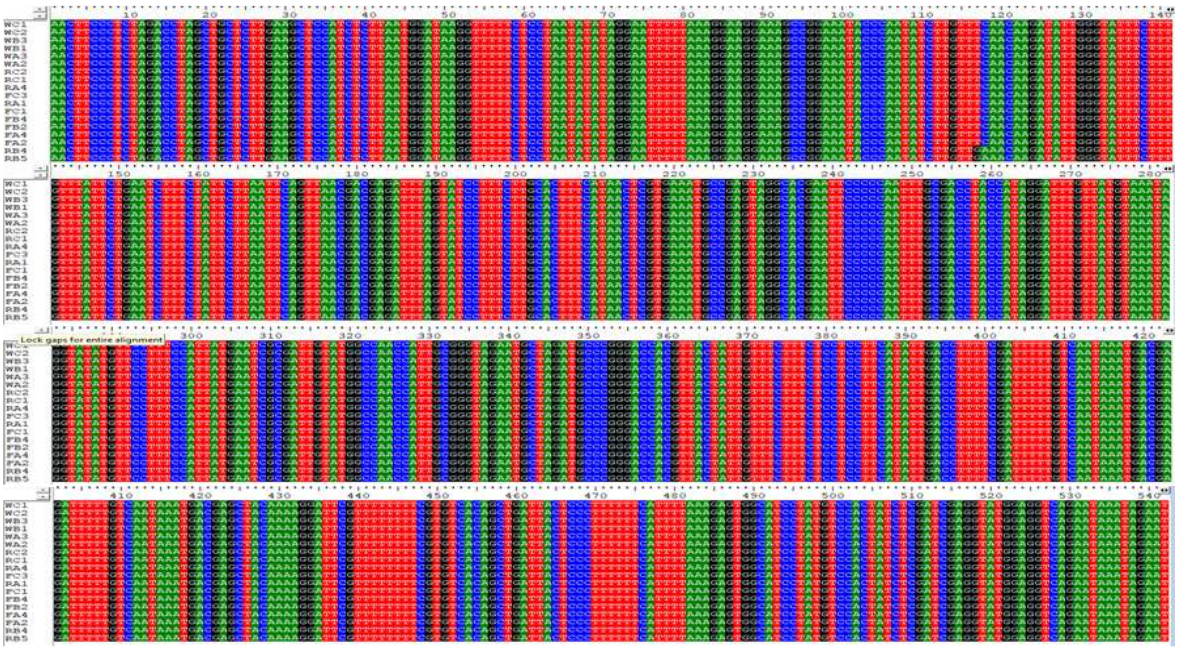


Figure 3. Complete sequence alignment of all *E. indica* samples from three habitat types- farm, roadside, and wasteland.

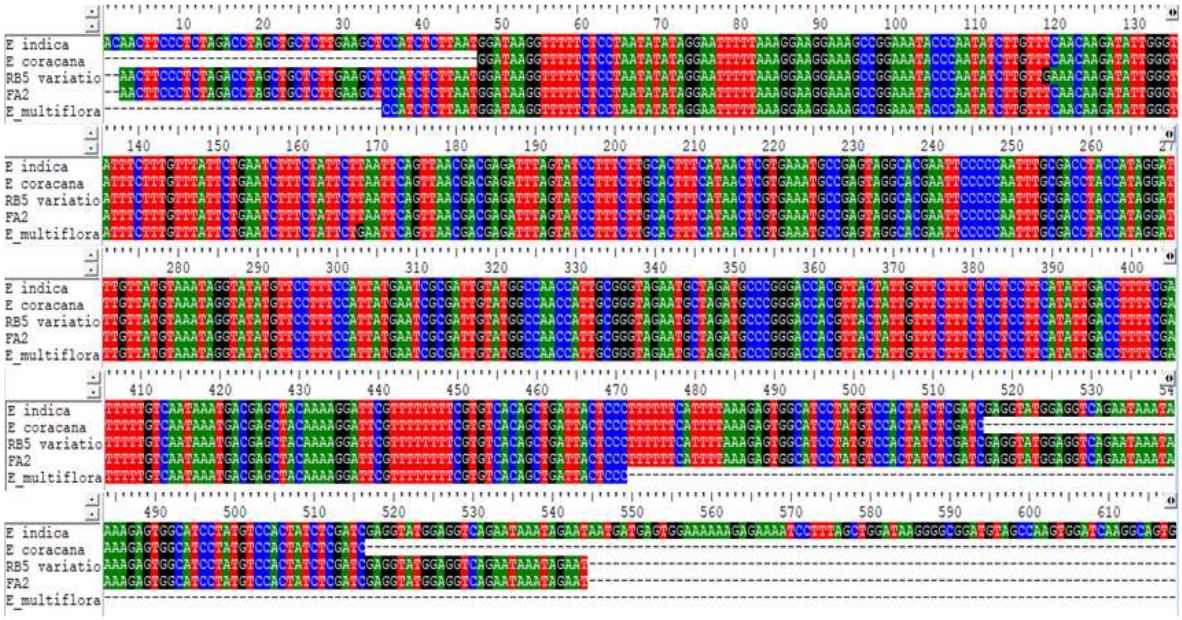


Figure 4. Multiple sequence alignment of three DNA sequences of *Eleusine* genus from Genbank and two haplotypes of *E. indica* from the present study. These GenBank sequences were selected as representative members of the genus *Eleusine* to enable comparison of both intra- and interspecific variation in the psbA–trnH region. *E. indica* (MH197403.1) served as a species-level reference, while *E. coracana* (OP903961.1) and *E. multiflora* (HQ876958.1) were included to represent closely and more distantly related congeners for assessing genetic divergence within the genus.

The genetic variation observed among *E. indica* populations from farm, roadside, and wasteland habitats was extremely low, indicating a high level of sequence similarity across all sampled environments. Overall, no substantial

sequence divergence was detected among individuals, and most samples shared identical psbA–trnH sequences. The only detectable variation occurred at nucleotide positions 117 and 118, where substitutions were observed exclusively in RB4 and RB5 from the roadside habitat. These substitutions resulted in the formation of two haplotypes: Haplotype 1, representing the majority of samples across all three habitats, and Haplotype 2, restricted to RB4 and RB5. All individuals from farm, roadside, and wasteland habitats clustered within Haplotype 1, suggesting minimal genetic differentiation and no clear association between habitat type and genetic structuring. Comparison with GenBank sequences of *Eleusine indica* (MH197403.1; Jiang *et al.*, 2019), *E. coracana* (OP903961.1; Jayalakshmi and Ganesh, 2023), and *E. multiflora* (HQ876958.1; Anderson *et al*., 2011) revealed limited polymorphism across the genus (Figure 4). The presence of only two haplotypes further supports a low level of intraspecific variation within the studied populations.

To further contextualize the genetic relationships, multiple sequence alignment was performed by comparing the two haplotypes identified in this study with three additional *Eleusine* sequences retrieved from GenBank. The aligned sequences ranged in length from 436 bp to 618 bp, reflecting interspecific variation within the genus. Across the alignment, only a few polymorphic sites were detected at positions 119, 120, and 168. These substitutions mainly involved purine–pyrimidine transversions, confirming that the observed variation was limited and largely conserved within the genus *Eleusine*. Overall, the nucleotide sequences across all five samples showed high similarity, indicating strong genetic conservation and low divergence at both intraspecific and interspecific levels.

This low diversity between individuals within the same species may be explained by local adaptation. Those who fit into their surroundings live longer and can reproduce well which passes on similar genes to the next generation. Some of these sites where sequence variation exists may be SNPs that do not impact an organism's traits. Such neutral changes can persist within populations without impacting individual fitness, thereby contributing to the overall genetic similarity among individuals. Only at three specific sites did minor transversions appear when the haplotypes from this study were compared to three other sequences of *Eleusine* species found on GenBank which are *E. indica, E. coracana* and *E. multiflora*. The lengths of the sequences in this study ranged from 436 to 618 bp. The tightness in sequence difference among species in this genus is a sign that *E. indica* does not have very diverse genes. This was in agreement with earlier reports that *E. indica* and *E. coracana* both come from the same genetic family and that *E. indica* is *E. coracana*'s maternal ancestor (2n = 4x= 36), known as finger millet. *E. multiflora* appears to hold an intermediary spot between *Eleusine* and *Acrachne* which could explain why it is not as similar to *E. indica*.

The limited performance of the *psbA-trnH* IGS marker may be due to the region's inherent lack of variability. While this barcode is often effective in angiosperms, its low diversity in *E. indica* restricts its ability to differentiate individuals of the same species. As Degtjareva *et al.* (2012) found, the marker was unable to tell apart species in other groups of plants as well. The limited amount of reference sequences on public databases such as GenBank, also makes it hard to do detailed analysis and proper identification of new species. Owing to the lack of more

sequences, this study allows for only limited interspecific investigation among *E. indica, E. coracana* and *E. multiflora*. It is harder to compare because the *psbA-trnH* region can vary a lot in length, from 150 to 905 bp in angiosperms. In this study, *E. multiflora* had a shorter sequence (436 bp) than the others, which made it more difficult to interpret genetic differences within the species.

Eco-reproductive factors might also contribute to the low genetic diversity observed in *E. indica* populations. Disturbances caused by humans along with disturbed habitats often lead to fewer individuals in a group and limits how genes move between them, possibly causing bottlenecks. In small and isolated habitats, genetic drift and inbreeding are more pronounced, often resulting in reduced genetic diversity. In addition, *E. indica* tends to reproduce itself with only self-fertilization. Since pollination occurs by itself, plants are sure to reproduce and do well in different places, though this reduces the chance of genetic variation. This investigation reveals that *E. indica* populations from diverse habitats have little genetic difference, as shown by sequence analysis of the *psbA-trnH* IGS region. Although the maker indicates that genes are evenly distributed and gaps between species are small, the low resolution it provides explains why using multi-locus or more variable markers in future work is necessary to see a complete picture.

CONCLUSIONS

Reproductive traits of *E. indica* such as spike length, peduncle length, and seed length varied significantly across farm, roadside, and wasteland habitats in Kampar, Perak. Roadside populations had shorter spikes, might be due to pollution and environmental stress. Peduncle and seed length differences were linked to plant height and resource availability. These results show that habitat conditions strongly influence reproductive traits. Genetic analysis using *psbA-trnH* IGS showed minor differences among habitats. Low genetic diversity within the *Eleusine* genus suggests close relationships between species. Further studies using more markers and reproductive traits are needed for an advanced study. Overall, the findings indicate that environmental conditions have a greater influence on reproductive trait variation than on genetic differentiation among *E. indica* populations in the study area. The observed morphological plasticity may contribute to the successful adaptation and persistence of *E. indica* across habitats with different disturbance levels. The low level of genetic variation detected using the psbA–trnH marker suggests that this chloroplast region may have limited resolution for assessing intraspecific diversity in *E. indica*. Therefore, the use of additional molecular markers, larger sample sizes, and broader geographic sampling is recommended to provide a more comprehensive understanding of the genetic structure, adaptation, and evolutionary potential of *E. indica* populations.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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