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Grain traits contributing to resistance to *Sitophilus oryzae*: A comparative analysis of 17 wheat genotypes

Jullia Parsons [a], Jordi Riudavets [a,*], Milica Škorić [b], Sanja Mikić [b], Radenka Kolarov [c], Dejan Prvulović [c], Snežana Tanasković [d], Sonja Gvozdenac [b]

[a] IRTA, Sustainable Plant Protection, IRTA Cabrils, Ctra. Cabrils km 2, Cabrils, Barcelona, 08348, Spain
[b] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, Novi Sad, 21000, Serbia
[c] Faculty of Agriculture University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, 21000, Serbia
[d] Faculty of Agronomy Čačak, University of Kragujevac, Carala Dušana 34, Čačak, 32303, Serbia

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ABSTRACT

Stored wheat suffers significant losses due to various storage pests, with *Sitophilus oryzae* being one of the most destructive. With limited plant protection products available, it is important to explore host plant resistance as a valuable tool in pest control. Wheat genotypes exhibit varying levels of resistance or susceptibility to *S. oryzae*, and identifying resistance-associated traits may contribute to improved resilience against storage pests. In this study, we evaluated the resistance and susceptibility of 17 wheat genotypes cultivated in Serbia, ranging from traditional landraces to contemporary cultivars, to *S. oryzae*, with the aim of identifying grain traits associated with reduced susceptibility and developing an optimal ideotype. Our approach combined grain phenotypic, biochemical, and physical traits with insect bionomic traits. The results showed that historical cultivars and landraces generally exhibited higher resistance to *S. oryzae* than most modern genotypes. After four months of infestation, the most resistant genotypes, including Garulja, Novosadska rana 2, Bankut 1205, and Crnozrna, showed low progeny production (19–33 individuals) and minimal grain consumption (0–3%), whereas the most susceptible modern cultivars, Dunav, Novosadska 100, and Libellula, had 188–247 individuals and 70–89% grain consumption. Reduced susceptibility was associated with darker grain pigmentation, higher protein and gluten contents, and selected biochemical traits, particularly flavonoids, which were negatively correlated with insect development. Together, these traits form a biochemical and physical profile linked to reduced susceptibility. This study provides a comprehensive assessment and new insights into trait associations linked to reduced susceptibility and offers a foundation for breeding wheat ideotypes with improved post-harvest protection.

1. Introduction

Wheat (*Triticum* spp., Poaceae) is one of the world's most important cereal crops and has been a dietary staple for thousands of years. Today, more than 2.5 billion people rely on wheat as a primary food source, with global production exceeding 800 million tons annually (FAOSTAT, 2025). The European Union is one of the world's largest wheat producers, with production figures in the range of over 110 million tons annually for common wheat and spelt (Eurostat, 2025). Serbia ranks among the top 20 wheat-producing countries, with an estimated production of around 3 million tons in 2024 (Statistical Office of the Republic of Serbia, 2024). However, during the postharvest period, wheat grains are threatened by insect pests that lead to qualitative and quantitative losses, making wheat storage challenging. Worldwide, approximately 15% or 25 million tons of wheat is lost during the storage phase (FAO, 2019). Among the various insect pests affecting stored wheat, the rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), is considered one of the most destructive. Although commonly associated with rice due to its scientific name, *S. oryzae* is a significant polyphagous primary pest of stored products, infesting a wide range of durable grain commodities, including wheat, barley, maize, sorghum, paddy rice and other cereal by-products (Athanassiou et al., 2017; Bhanderi et al., 2015; Doherty et al., 2023; Gvozdenac et al., 2020). Despite its broad host range, previous studies have shown that *S. oryzae* exhibits a strong preference for wheat (Athanassiou et al., 2008; Bohinc et al., 2024).

* Corresponding author.
E-mail address: jordi.riudavets@irta.cat (J. Riudavets).

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In storage environments, *S. oryzae* is particularly damaging because it thrives under common storage conditions of 25–30 °C and 60–65% relative humidity (Aslam et al., 2017; Lu et al., 2024). During its larval phase, *S. oryzae* primarily feeds internally on the grain endosperm, compromising seed germination, reducing protein and vitamin content, and consuming a substantial portion of the total grain weight during development (Mehta et al., 2021; Rita Devi et al., 2017). As an adult, it further diminishes grain quality by reducing carbohydrate content (Majd-Marani et al., 2023). Beyond the direct damage it causes, as a primary pest, *S. oryzae* creates opportunities for secondary pests (external feeders) and pathogenic organisms to further deteriorate the grain.

Traditionally, this insect pest has been managed by chemical control methods. However, the heavy reliance on a limited spectrum of approved plant protection products (insecticides) has led to an increase in insect populations that are resistant to specific compounds, thus reducing their overall efficacy (Nayak et al., 2019). As a result, the chemical control efficacy and insecticide availability have diminished, creating demand for alternative strategies to manage *S. oryzae* infestations. One of the most promising solutions within integrated pest management is the use of inherently resistant genotypes. Although *S. oryzae* can develop and thrive on a wide range of hosts, its behavior is influenced by various physical and chemical factors that affect its host selection, feeding patterns, and development (Abedi et al., 2024). Therefore, understanding the traits that influence host suitability and confer resilience is essential for developing effective breeding strategies that ensure resistance not only under field conditions but also during the postharvest period, when grains remain vulnerable to infestation.

Key factors contributing to grain resistance, as suggested by Bergvinson and García-Lara (2004), are biochemical and physical traits, which often interact and overlap. Biochemical resistance in wheat is evident through elevated levels of phenolic compounds, which disrupt pest digestion by reducing nutrient availability or introducing toxic effects (Kaur et al., 2014; Mishra et al., 2015). These compounds interfere with the pests' ability to digest and absorb essential nutrients, ultimately making the grain less suitable for their survival. Phenolic acids, flavonoids, and tannins have been reported to contribute to the plant's defense mechanisms (Dar et al., 2024; Xu et al., 2021). These compounds, especially when present in higher concentrations, have been associated with resistance to *Sitophilus* pests (Kordan et al., 2019). The genes responsible for synthesizing these compounds are integral to the chemical defense of the grain, helping reduce damage from pests (Kumar et al., 2020; Shen et al., 2022). In addition to phenolic compound content, the nutritional composition of wheat may also play a role in influencing pest host selection and development. Grains with higher carbohydrate content, particularly starch, as the primary energy reserve of the grain, may provide a suitable food source for *S. oryzae*, as adults primarily feed on the endosperm where these reserves are concentrated (Arya et al., 2025). Likewise, higher levels of proteins and vitamins in the germ may support larval development and contribute to greater progeny production, since larvae feed mainly on the germ and depend on its nutritive value (Gvozdenac et al., 2020; Kosewska et al., 2024; Yılmaz and Bayram, 2026).

Traits such as moisture content, grain hardness, grain color, and grain size also contribute to biophysical resistance of grains. For example, lower moisture levels can create an unfavorable environment for *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), reducing their development and survival (Zhang et al., 2020). Additionally, harder grains can physically damage the pests' mouthparts and hinder the larval feeding efficiency of *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae), making it more difficult for them to consume and process food (D'Isita et al., 2024). Certain grain colors may deter pests or reduce their attractiveness, further contributing to resistance. Yan et al. (2023) discovered that darker-colored wheat grains tend to exhibit higher levels of resistance against *S. oryzae,* potentially due to the presence of phenolic compounds that deter pest feeding. The grain size might also influence the preference of *S. oryzae*, with larger grains being more attractive as a host.

Biochemical and biophysical resistance in post-harvest wheat grains may be expressed through antixenosis and/or antibiosis. Antixenosis deters pests from feeding or laying eggs through physical traits or repellent compounds, such as specific organic volatile compounds, making the grain less attractive for infestation. Antibiosis affects pest biology after feeding or oviposition, leading to slower growth, lower survival, reduced reproduction, or delayed development (Wiseman, 1985, 1994). Since the present study used a no-choice bioassay, the results mainly reflect genotype effects on insect development and grain consumption, which are more closely related to antibiosis-related resistance.

This study aimed to identify grain traits associated with wheat resistance to *S. oryzae* using 17 selected wheat genotypes cultivated in Serbia, including modern and historical cultivars, landraces, and a breeding line. To achieve this aim, the study integrates detailed grain phenotyping including morphological traits such as grain hardness, moisture, and color, alongside biochemical analysis that encompassed nutrient content, phenolic compounds content (total phenols, flavonoids, and tannins) and antioxidant activity. The collected phenotypic data are combined with insect bioassay results, assessing progeny production and grain consumption (%).

This integrative analysis aimed to identify grain traits associated with reduced susceptibility to *S. oryzae* under no-choice conditions and to support the identification of wheat traits useful for breeding genotypes with improved post-harvest resistance. By improving understanding of genotype-dependent variation in susceptibility to *S. oryzae*, this research may contribute to more sustainable grain protection under storage conditions and support future breeding of resistant genotypes better adapted to increasing pest pressure.

2. Materials and methods

2.1. *Sitophilus oryzae* population rearing

The initial population of *S. oryzae* was obtained from the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad. The population was maintained on commercial bread-wheat grains, variety Pobeda. Before use, grains intended for colony maintenance were cleaned, sieved to remove dust and broken grain particles, and sterilized at 60 ± 5 °C for 8 h to eliminate possible visible or hidden infestation by insects or mites. Adult weevils were transferred to rearing containers containing healthy, uninfested wheat grains, and the containers were covered with muslin cloth to allow aeration. Every two weeks, newly emerged adults from each rearing cycle were sieved and transferred to fresh rearing containers. Adults emerging from these cultures were used for the experiments. The population was maintained throughout the experimental period under laboratory conditions at 27 ± 2 °C, 70% RH, and a 16:8 h light photoperiod.

2.2. *Wheat genotypes*

A total of 17 wheat genotypes were used in these experiments. The genotypes represent selected genotypes developed through different improvement methods and classified into four cultivar groups representing distinct stages of breeding history (Table 1). Modern cultivars consisted of recently developed commercial varieties bred for high yield and agronomic performance. Historical cultivars included older commercial varieties that were widely grown in the first half of the 20th century but are no longer cultivated. Landraces were traditional farmer-maintained varieties that originated through local selection and are genetically more diverse, representing pre-breeding material. The breeding group contained an advanced experimental line that has not been released as commercial variety. Together, these categories represent the full spectrum of wheat improvement, from traditional landraces to contemporary elite cultivars where 15 of these belonged to bread

Table 1
Wheat genotypes grouped by different cultivar types.

Name	Type	Species	Germplasm identifiers
Novosadska 100	modern cultivar	*Triticum aestivum*	10.18730/ZAGE9
Mačvanka	modern cultivar	*Triticum aestivum*	10.18730/ZM14W
Dunav	modern cultivar	*Triticum aestivum*	10.18730/ZAGB6
Panonija	modern cultivar	*Triticum aestivum*	10.18730/ZAGPH
Novosadska crvena	modern cultivar	*Triticum aestivum*	10.18730/ZAG1~
Novosadska rana 2	modern cultivar	*Triticum aestivum*	10.18730/ZM10R
Libellula	modern cultivar	*Triticum aestivum*	10.18730/ZAGHC
Novosadska 32	modern cultivar	*Triticum aestivum*	10.18730/ZAFZZ
Bačka	modern cultivar	*Triticum aestivum*	10.18730/ZAG4U
Rumska crvenka	historical cultivar	*Triticum aestivum*	10.18730/ZAF77
Bezostaya 1	historical cultivar	*Triticum aestivum*	10.18730/ZAGGB
Bankut 1205	historical cultivar	*Triticum aestivum*	10.18730/ZAG2$
Crnozrna	historical cultivar	*Triticum aestivum*	10.18730/ZAFXX
Stara banatka	landrace	*Triticum aestivum*	10.18730/ZAG0*
Garulja	landrace	*Triticum aestivum*	10.18730/ZAEMS
Velja pšenica	landrace	*Triticum turgidum*	10.18730/ZAGRK
NSD 3/92	Breeding line	*Triticum durum*	10.18730/ZM16Y

wheat (*Triticum aestivum*) and two were tetraploid wheat (*Triticum durum* and *Triticum turgidum*).

2.3. No-choice insect bioassay

A no-choice test was conducted in Petri dishes with a diameter of 90 mm to evaluate the susceptibility or resistance of 17 wheat genotypes to *S. oryzae* infestation. Susceptibility/resistance was assessed based on progeny production in each genotype after one, two, three, and four months of exposure, as well as grain consumption (%) after four months. Each Petri dish contained 50 g of intact grains from a specific wheat genotype, with three replicates per genotype. Twenty young adult weevils, up to 7 days old, were introduced into each dish, with an assumed 1:1 sex ratio. To assess progeny production, all adult weevils present in each Petri dish, including both live and dead individuals, were counted once per month for four consecutive months. Because the initial adults were not removed after oviposition, the 20 introduced adults were subtracted from each monthly total count to estimate the cumulative number of newly emerged adults at each observation time. In addition, grain consumption was measured at the final observation after four months by weighing the remaining wheat grains after removing dust, flour, insects, and insect body parts. Grain consumption was calculated by subtracting the remaining grain weight from the initial 50 g offered and expressing the result as a percentage.

2.4. Morphological and physical traits of wheat grains

Morphological characterization of the wheat grains was performed according to the UPOV descriptor (UPOV, 2012).

2.4.1. Grain hardness

Grain hardness was evaluated based on the external force (N) required to crush the grain. Measurements were performed at the Laboratory for Biosystem Engineering, Faculty of Agriculture, University of Novi Sad, using a TMS-Pro Advanced texture analyzer (Food Technology Corporation, USA). Three grains were measured per genotype. A load cell with a capacity of 500 N was used in the experiment. The pre-test speed was 60 mm/min, and the test speed was 30 mm/min. The probe displacement was set to 2 mm.

2.4.2. Colorimeter analysis

Wheat grains were subjected to instrumental color assessment using a colorimetric device (Konica Minolta CR-400) and the CIELAB color model. Five grains were measured per genotype. The device provides the lightness coordinate (L*; ranging from 0 = perfect black to 100 = perfect white), redness coordinate (a*; from green to red), and yellowness coordinate (b*; from blue to yellow), following the standard CIELAB color space conventions.

2.5. Nutrient composition of wheat grains

The nutrient composition of wheat grain was analyzed using the DA 7250™ Diode Array Near-Infrared Analysis System (PerkinElmer, Inc., Waltham, Massachusetts, USA). The analysis of 17 wheat genotypes was carried out by means of a rapid and accurate indirect method. The maximum analyzed surface area reached up to 108 cm^2. The instrument operated in measurement mode using down-view reflectance or transflectance. The detector wavelength range was 900–1700 nm, with an operational range of 950–1650 nm, and a resolution of <0.05 nm. The detector type was a thermoelectrically cooled, 256-pixel, high-detectability indium gallium arsenide (InGaAs).

Grains of each genotype were placed into small trays, and the surface was leveled using a ruler with gentle shaking. On average, 100-150 g of grain per genotype was used for analysis. Each measurement was performed in five replicates. Two tray types were applied in the measurements: rotating and static. The following traits were measured: moisture, protein, wet gluten, dry gluten, and starch.

2.6. Biochemical analysis of wheat grains

Biochemical analysis encompassed the determination of phenolic compound content and antioxidant activity of grains of 17 wheat genotypes.

2.6.1. Grain preparation for biochemical analysis

Wheat grains were ground to a fine powder. For ethanol extraction, 1 g of ground grain per genotype was homogenized with 5 mL of 70% ethanol, and the extracts were kept in the dark at room temperature for 24 h with occasional stirring. Each biochemical assay was performed in six replicates per genotype.

2.6.2. Phenolic compounds content

Total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu (FC) colorimetric method, which measures the reducing capacity of phenolic compounds toward the FC reagent (Hudz et al., 2019; Wootton-Beard et al., 2011). After reaction in alkaline medium, absorbance was recorded at 730 nm, and phenolic concentration was calculated from a quercetin standard curve.

Total tannin content was determined using a polyvinylpolypyrrolidone (PVPP) binding assay, where extracts were incubated with insoluble PVPP to adsorb tannins, followed by Folin–Ciocalteu measurement of non-tannin phenolics. Tannin content was calculated as the difference between total phenolics and PVPP-treated phenolics and expressed as mg quercetin equivalents per gram of dry matter (mg QE g^{-1} dm). Total flavonoid content was measured using an aluminium chloride ($AlCl_3$) colorimetric assay, in which flavonoids form a yellow complex with maximum absorbance at 430 nm (Martono and Muninggar, 2020). Flavonoid concentration was

calculated from a quercetin standard curve and expressed as quercetin equivalents per gram of dry matter (mg QE g^{-1} dm).

2.6.3. Antioxidative activity

Total antioxidant activity (TAA) was determined using the phosphomolybdenum colorimetric assay, in which antioxidants reduce a molybdenum (VI) reagent to a green complex measured at 695 nm (Prieto et al., 1999). Results were calculated from a Trolox standard curve.

DPPH radical scavenging activity was determined according to Floegel et al. (2011), with slight modifications, based on the reduction of the purple DPPH• radical to its yellow reduced form. Absorbance was measured at 517 nm after 30 min of incubation in the dark, and results were calculated from a Trolox standard curve.

ABTS radical cation scavenging activity was determined according to Re et al. (1999). The ABTS•+ reagent was generated by reacting ABTS with potassium persulfate in the dark, diluted to an absorbance of approximately 0.7 at 734 nm, and mixed with the extracts. Absorbance was measured at 734 nm after 2 h of incubation in the dark, and results were calculated from a Trolox standard curve.

Ferric reducing antioxidant power (FRAP) was measured using the Fe^{3+}–TPTZ reduction assay, in which antioxidants reduce the Fe^{3+}–TPTZ complex to the blue Fe^{2+}–TPTZ form with absorbance read at 593 nm (Lim and Lim, 2013). Results were calculated from a Trolox standard curve.

2.7. Statistical analysis

A generalized linear model (GLM) with a negative binomial error distribution and log link was used to analyze both the total number of *S. oryzae* individuals after each observation period (progeny production) and the percentage of grains consumed. For each sampling period (month), a separate model was fitted to test differences among genotypes. Additionally, a separate model was conducted to test differences among cultivar types; however, this analysis was interpreted cautiously because cultivar-type groups were unbalanced and the breeding-line category included only one genotype. The negative binomial family was chosen to account for overdispersion in the data. Overall effects were evaluated with likelihood-ratio χ^2 tests, and when significant ($\alpha = 0.05$), pairwise comparisons of estimated marginal means were performed with Tukey's adjustment for multiple testing.

Grain hardness, nutrient composition parameters (moisture, protein, dry and wet gluten, and starch), and biochemical parameters (phenols, tannins, flavonoids, total antioxidant activity, DPPH, ABTS, and FRAP) were analyzed separately from the insect bioassay data. Differences among wheat genotypes were evaluated separately for each parameter using one-way analysis of variance (ANOVA), with genotype as the fixed factor. Pairwise mean separation among genotypes was performed using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Results are presented as mean ± standard error (SE), followed by different letters indicating significant differences among genotypes.

Pearson correlation coefficients were calculated among all grain quality, biochemical, and resistance traits using the rcorr function in the Hmisc package (R). The correlation matrix was visualized with ggcorrplot as a lower-triangle heatmap.

Grain color was characterized in CIELAB space using the L*, a* and b* coordinates for each wheat genotype. For visualization, mean L*, a* and b* values across replicates were calculated per genotype and plotted in a three-dimensional CIELAB scatterplot in R. Each point represents one genotype, identified by a distinct plotting symbol, while an adjacent colored spot shows the corresponding perceived color, reconstructed from the CIELAB coordinates using the colorspace package.

All statistical analyses and data visualizations were performed using R version 3.5.1 (R Core Team, 2018) within RStudio version 1.1.463.

3. Results

3.1. No-choice insect bioassay

There were significant differences in progeny production of *S. oryzae* among the 17 wheat genotypes at each observation period, indicating genotype-dependent differences in susceptibility. Differences were also detected among cultivar types; however, because cultivar-type groups were unbalanced, these results are interpreted cautiously and the main interpretation is based on genotype-level comparisons.

After the first month, the number of *S. oryzae* individuals per genotype ranged from 12 to 20, likely because many individuals were still developing inside the wheat grains and could not be counted at that time.

After two months, progeny production significantly differed among genotypes ($\chi^2 = 42.19$, df = 16, $p < 0.001$) (Fig. 1). Among cultivar types, progeny production was higher in modern cultivars than in historical cultivars ($\chi^2 = 17.52$, df = 3, $p < 0.001$). Among modern cultivars, Novosadska 100 was the most susceptible genotype, with 86 individuals recorded, whereas Novosadska rana 2 showed lower progeny production, with 23 individuals. Historical cultivars remained relatively less susceptible, with 14–42 individuals recorded. Landraces also showed relatively low progeny production, with counts below 41 individuals. In contrast, the breeding line NSD 3/92 showed relatively high susceptibility, with 85 individuals recorded. Overall, after two months of infestation, Bankut 1205 was among the most resistant genotypes, whereas Novosadska 100 was the most susceptible.

After the 3-month assessment, significant differences among genotypes were observed ($\chi^2 = 53.05$, df = 16, $p < 0.001$). The modern cultivar group again showed higher susceptibility than the historical cultivars ($\chi^2 = 22.59$, df = 3, $p < 0.001$). Within the modern cultivars, Dunav was the most susceptible genotype, with 179 individuals recorded, whereas lower progeny production was observed in Novosadska 32 (38 individuals), Panonija (51 individuals), and Novosadska rana 2 (26 individuals). Among historical cultivars, the number of individuals remained low and did not exceed 42. The number of individuals in the landraces also remained relatively low, particularly in Velja pšenica and Garulja, with 32–41 individuals. In contrast, the breeding line NSD 3/92 showed relatively high susceptibility, with 94 individuals recorded. Overall, after three months, the most resistant genotypes were Bankut 1205 and Novosadska rana 2, whereas the most susceptible were Dunav and Novosadska crvena.

After four months, there were significant differences among genotypes ($\chi^2 = 81.86$, df = 16, $p < 0.001$). When cultivar types were compared, modern cultivars showed higher susceptibility than historical cultivars and landraces ($\chi^2 = 17.55$, df = 3, $p < 0.001$). Within the modern cultivars, Dunav, Novosadska 100, and Libellula were the most susceptible genotypes, with 188–247 individuals recorded after four months. In contrast, in Novosadska 32, Panonija, and Novosadska crvena, the number of newly emerged weevils ranged from 76 to 80, while Novosadska rana 2 was the least susceptible modern cultivar, with 23 individuals. Among the historical cultivars, Rumska crvenka was the most susceptible (88 individuals), whereas Crnozrna was the least susceptible based on progeny production (33 individuals). Landraces also showed variability, with Stara banatka (92 individuals) and Velja pšenica (70 individuals) showing higher susceptibility than Garulja (19 individuals). The breeding line NSD 3/92 again showed high susceptibility, with 94 individuals recorded. Overall, after four months, the most resistant genotypes were Novosadska rana 2, Bankut 1205, Crnozrna, and Garulja, whereas the most susceptible genotypes were Dunav, Novosadska 100, and Libellula.

Grain consumption (%) was assessed after four months of *S. oryzae* infestation. Differences among genotypes were significant ($\chi^2 = 103.94$, df = 16, $p < 0.001$) (Fig. 2). Significant differences were also found among cultivar types, with modern cultivars and the breeding line showing higher grain consumption than historical cultivars and

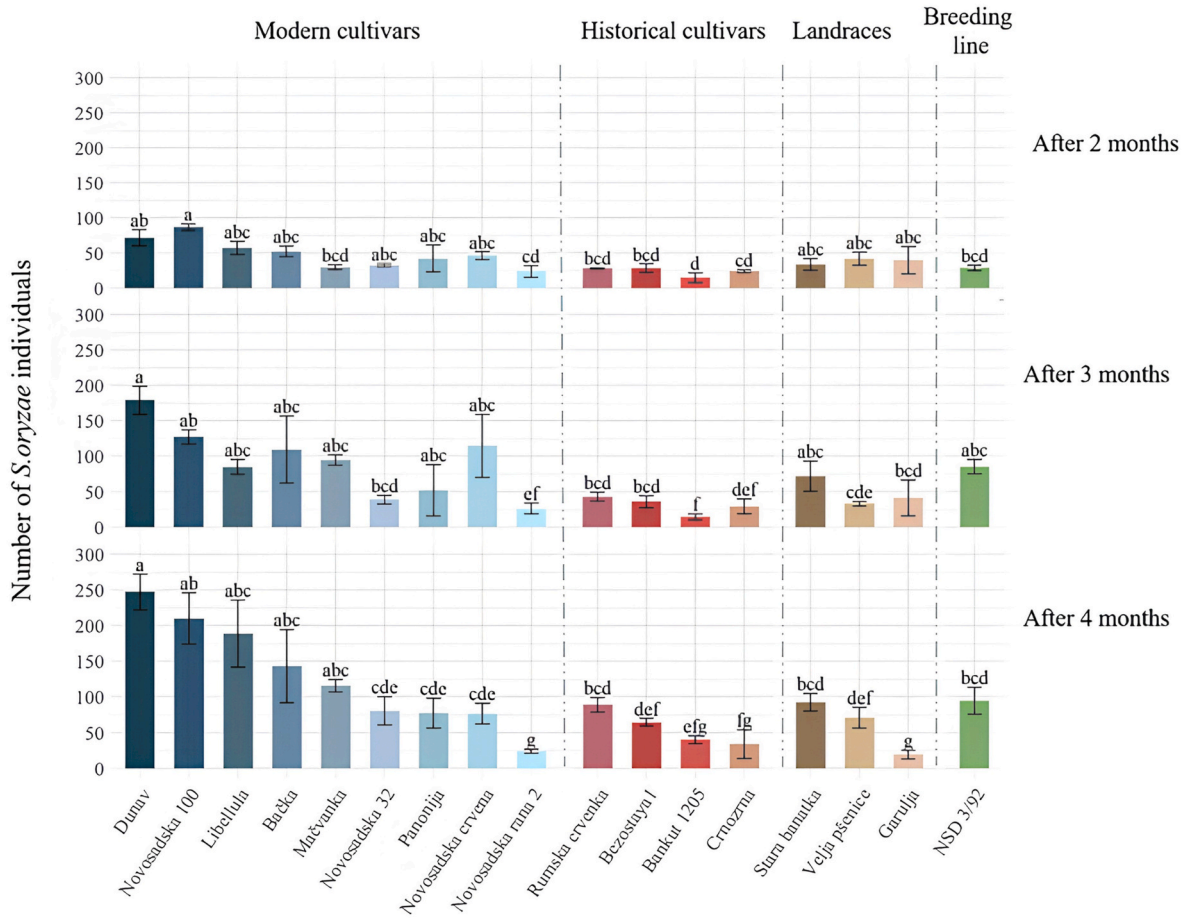

Fig. 1. Number of *S. oryzae* weevils on 17 wheat genotypes after 2, 3, and 4 months of infestation (mean ± SE). Different lower-case letters indicate significant differences among genotypes (Tukey's test, $p < 0.05$).

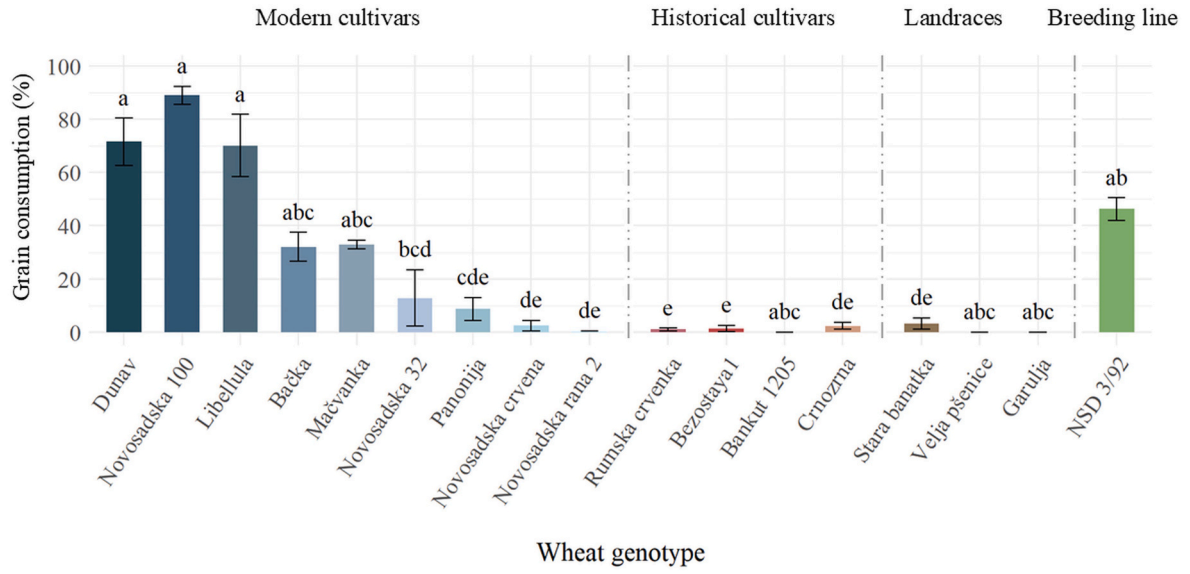

Fig. 2. Grain consumption (%) after 4 months of *S. oryzae* infestation (mean ± SE). Bars with different lower-case letters indicate significant differences among genotypes (Tukey's test, $p < 0.05$).

landraces ($\chi^2 = 49.39$, df = 3, $p < 0.001$). Among the modern cultivars, Novosadska 100 was the most heavily consumed (89%), while Dunav and Libellula also showed high grain consumption (70% and 71%, respectively). In contrast, Novosadska 32, Panonija, Novosadska crvena, and Novosadska rana 2 were the least consumed modern cultivars, with only 0–12% grain consumption.

The proportion of consumed grains among historical cultivars and landraces remained extremely low (0–3%), indicating low susceptibility

to this insect species. In the breeding line NSD 3/92, grain consumption was 46%, indicating high susceptibility. In general, the least consumed genotypes belonged to the historical cultivar and landrace groups, whereas the most consumed genotypes were Novosadska 100, Dunav, and Libellula.

3.2. Morphological and physical traits of wheat grains

3.2.1. Grain hardness

Grain hardness varied significantly among genotypes, ranging from 48.87 N in Novosadska crvena to 306.33 N in Libellula, indicating that Libellula had the hardest grain texture. Relatively high hardness values were also observed in Velja pšenica (135.67 N) and Bezostaya 1 (128.17 N), whereas softer grains were recorded in Novosadska 32 and Dunav (Table 2).

3.2.2. Colorimetric analysis

Grain color variation among genotypes was evaluated in the CIELAB color space using the coordinates L* (lightness), a* (green–red axis), and b* (blue–yellow axis). Most genotypes exhibited intermediate to high L* values (approximately 48–58), indicating light to medium-light kernel coloration, accompanied by moderate a* and b* values. The cultivars Dunav, Libellula, NSD 3/92, Panonija, and Novosadska 32 showed the highest L* values within the analyzed set, corresponding to the lightest kernel appearance. In contrast, the landrace genotypes Crnozrna and Velja pšenica exhibited the lowest L* values (approximately 35), indicating darker kernel coloration relative to the other genotypes (Fig. 3).

3.3. Nutritional composition of wheat grains

Moisture content differed significantly among genotypes [$F(16, 34) = 61.04$, $p < 0.001$]. The highest moisture content was recorded in Dunav (10.24%) and Mačvanka (10.21%), whereas the lowest value was observed in Crnozrna (7.83%), followed by Novosadska crvena (8.85%) (Table 2).

Protein content varied significantly among genotypes [$F(16, 34) = 75.40$, $p < 0.001$], ranging from 10.54% in Dunav to 14.75% in Garulja. Relatively high protein levels were also recorded in Stara banatka (14.40%), Mačvanka (14.19%), Bankut 1205 (14.18%), and Rumska crvenka (14.12%).

Dry gluten content differed significantly among genotypes [$F(16, 34) = 27.30$, $p < 0.001$], ranging from 8.93% in Dunav to 12.85% in Garulja. Relatively high dry gluten values were also recorded in Bankut 1205 (12.27%) and Stara banatka (12.06%), whereas lower values were observed in Novosadska 100 (9.91%) and Crnozrna (10.14%). Wet gluten content followed a similar pattern and also differed significantly among genotypes [$F(16, 34) = 39.62$, $p < 0.001$], ranging from 25.07% in Dunav to 36.69% in Garulja. Relatively high wet gluten levels were also observed in Stara banatka (34.37%), Bankut 1205 (34.36%), and Mačvanka (34.30%).

Starch content differed significantly among genotypes [$F(16, 34) = 24.11$, $p < 0.001$] and showed an inverse tendency compared with protein and gluten content. The highest starch content was recorded in Dunav (75.42%), followed by Novosadska 32 (74.38%) and Crnozrna (74.24%), whereas the lowest values were observed in Mačvanka (71.45%), Bankut 1205 (71.84%), and Garulja (71.88%).

Overall, Libellula was characterized by the highest grain hardness, while Garulja, Stara banatka, Bankut 1205, and Mačvanka showed relatively high protein and gluten contents. In contrast, Dunav combined high starch content with low protein and gluten levels, and Crnozrna had the lowest moisture content among the tested genotypes.

3.4. Biochemical analysis

There were no significant differences among genotypes for total phenols and tannins, whereas flavonoid content showed highly significant variation. Antioxidant activity parameters showed significant genotype effects for total antioxidant activity, ABTS, and FRAP, while DPPH values did not differ significantly among genotypes (Table 3).

3.4.1. Phenolic compound content

Regarding phenolic compounds, total phenol content ranged from 2.300 mg QE g^{-1} dm in Libellula to 3.999 mg QE g^{-1} dm in Novosadska crvena. Although numerically higher values were observed in Bankut 1205 (3.963 mg QE g^{-1} dm), Rumska crvenka (3.794 mg QE g^{-1} dm), and Panonija (3.711 mg QE g^{-1} dm), these differences were not statistically significant among genotypes [$F(16, 34) = 0.98$, $p = 0.497$].

Tannin content ranged from 0.542 mg QE g^{-1} dm in Mačvanka to 0.875 mg QE g^{-1} dm in Bezostaya 1, with numerically high values also observed in Crnozrna and Novosadska crvena. However, tannin content did not differ significantly among genotypes [$F(16, 34) = 1.39$, $p = 0.204$]. Flavonoid content ranged from 0.215 mg QE g^{-1} dm in Bačka and NSD 3/92 to the highest values in Stara banatka (0.469 mg QE g^{-1} dm) and Bankut 1205 (0.454 mg QE g^{-1} dm). Genotype had a significant effect on flavonoid content [$F(16, 34) = 5.03$, $p < 0.001$].

Table 2
Grain hardness, moisture, protein content, dry gluten, wet gluten, and starch content of grains from 17 wheat genotypes. Values are presented as mean ± standard error (SE). Different letters within a column indicate significant differences among genotypes according to Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. F values indicate the significance of the overall genotype effect: ns, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p < 0.001$.

Genotype	Hardness (N)	Moisture %	Protein %	Dry gluten %	Wet gluten %	Starch %
Dunav	66.33 ± 2.67cd	10.24 ± 0.16a	10.54 ± 0.46e	8.93 ± 0.23d	25.07 ± 1.59e	75.42 ± 0.42a
Novosadska 100	85.33 ± 8.76cd	9.55 ± 0.04b	11.41 ± 0.18d	9.91 ± 0.26cd	28.06 ± 0.32d	72.00 ± 0.21cd
Libellula	306.33 ± 19.10a	10.02 ± 0.02a	13.38 ± 0.04b	11.50 ± 0.03b	32.27 ± 0.07bc	72.63 ± 0.11cd
Bačka	73.33 ± 2.33cd	10.13 ± 0.08a	13.41 ± 0.06b	11.21 ± 0.02b	32.38 ± 0.24bc	72.71 ± 0.19c
Mačvanka	77.00 ± 6.56cd	10.21 ± 0.05a	14.19 ± 0.15a	11.79 ± 0.26b	34.30 ± 0.98a	71.45 ± 0.14d
Novosadska 32	65.30 ± 3.51cd	10.00 ± 0.06a	11.84 ± 0.08cd	10.65 ± 0.06c	27.83 ± 0.08d	74.38 ± 0.16 ab
Panonija	82.13 ± 5.29cd	9.92 ± 0.02a	12.57 ± 0.14c	11.53 ± 0.29b	30.22 ± 0.55c	72.03 ± 0.36cd
Novosadska crvena	48.87 ± 5.31d	8.85 ± 0.02c	11.47 ± 0.09d	10.29 ± 0.19c	26.40 ± 0.25de	73.62 ± 0.21b
Novosadska rana 2	104.23 ± 6.04bc	9.51 ± 0.05b	11.72 ± 0.09d	10.40 ± 0.18c	27.28 ± 0.12de	74.03 ± 0.09b
Rumska crvenka	81.37 ± 2.90cd	10.00 ± 0.03a	14.12 ± 0.05a	11.92 ± 0.05 ab	33.83 ± 0.05b	72.27 ± 0.16cd
Bezostaya1	128.17 ± 2.04b	9.48 ± 0.04b	13.03 ± 0.04b	11.67 ± 0.11b	31.60 ± 0.11bc	73.70 ± 0.23b
Bankut 1205	111.77 ± 0.50b	9.74 ± 0.02b	14.18 ± 0.05a	12.27 ± 0.11a	34.36 ± 0.06a	71.84 ± 0.11cd
Crnozrna	99.03 ± 8.69bc	7.83 ± 0.16d	11.80 ± 0.10d	10.14 ± 0.43c	31.30 ± 0.17bc	74.24 ± 0.32 ab
Stara banatka	76.23 ± 0.54cd	10.10 ± 0.04a	14.40 ± 0.05a	12.06 ± 0.03a	34.37 ± 0.04a	72.11 ± 0.08cd
Velja pšenice	135.67 ± 16.95b	9.74 ± 0.04b	12.87 ± 0.05b	11.02 ± 0.03b	31.43 ± 0.04bc	73.78 ± 0.19b
Garulja	93.17 ± 5.72c	9.75 ± 0.06b	14.75 ± 0.11a	12.85 ± 0.08a	36.69 ± 0.07a	71.88 ± 0.26cd
NSD 3/92	111.33 ± 6.69b	9.92 ± 0.14a	12.02 ± 0.11cd	11.45 ± 0.23b	31.03 ± 0.63c	73.55 ± 0.33b
F value	52.83***	61.04***	75.40***	27.30***	39.62***	24.11***

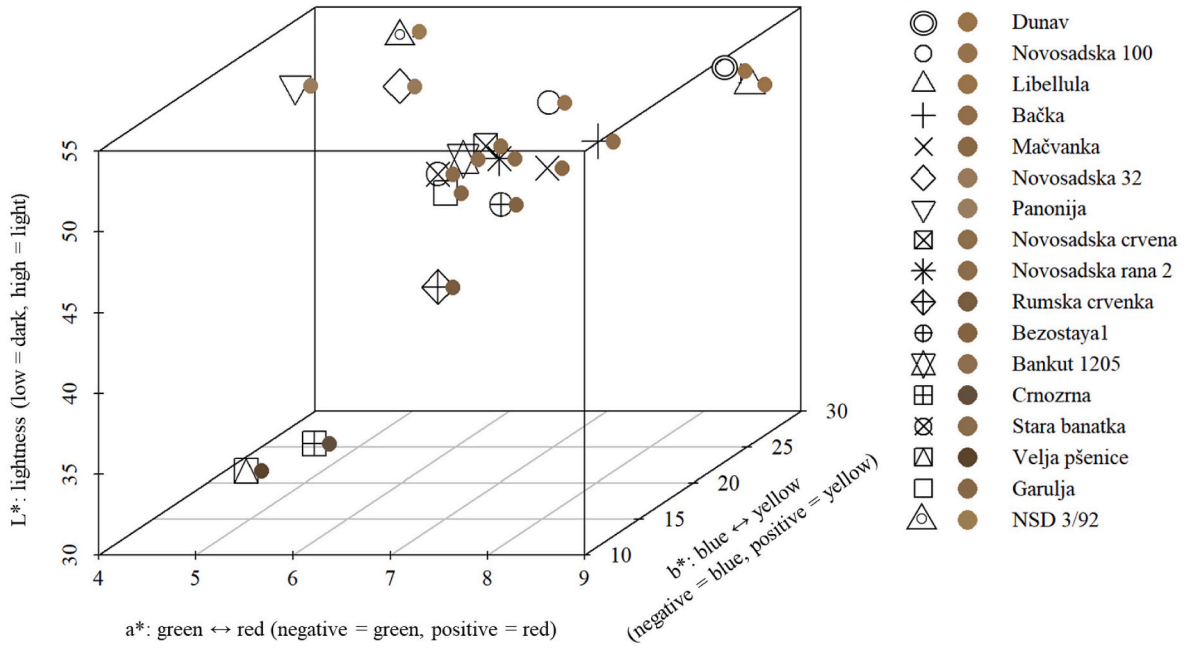

Fig. 3. Three-dimensional scatterplot of wheat grain color in CIELAB space (L*, a*, b*). L* represents lightness (low = dark, high = light), a* the green–red axis, and b* the blue–yellow axis. Each point corresponds to a genotype with their corresponding simulated color spot. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Phenolic compounds and antioxidant activity of the 17 wheat genotypes. Total phenols, tannins, and flavonoids are expressed as mg quercetin equivalents per gram of dry matter (mg QE g^{-1} dm). Total antioxidant activity (TAA), DPPH radical scavenging activity, ABTS radical cation scavenging activity, and ferric reducing antioxidant power (FRAP) are expressed as mg Trolox equivalents per gram of dry matter (mg TE g^{-1} dm). Values are presented as mean ± standard error (SE). Different letters within a column indicate significant differences among genotypes according to Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. F values indicate the significance of the overall genotype effect: ns, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p < 0.001$.

Genotype	Phenols	Tannins	Flavonoids	TAA	DPPH	ABTS	FRAP
Dunav	2.700 ± 0.451a	0.707 ± 0.051a	0.223 ± 0.004b	11.421 ± 0.777 ab	6.263 ± 0.627a	4.031 ± 0.306 ab	4.392 ± 0.088a
Novosadska 100	3.133 ± 0.318a	0.615 ± 0.092a	0.222 ± 0.002b	11.507 ± 0.755 ab	5.733 ± 0.279a	4.015 ± 0.112 ab	4.009 ± 0.366a
Libellula	2.300 ± 0.153a	0.595 ± 0.035a	0.217 ± 0.010b	12.018 ± 0.479 ab	6.951 ± 0.567a	3.091 ± 0.040b	3.600 ± 0.199a
Bačka	2.767 ± 0.088a	0.609 ± 0.065a	0.215 ± 0.005b	11.491 ± 0.830 ab	6.846 ± 0.480a	4.256 ± 0.372 ab	4.075 ± 0.190a
Mačvanka	3.000 ± 0.100a	0.542 ± 0.025a	0.221 ± 0.008b	12.576 ± 0.073a	5.406 ± 0.215a	3.987 ± 0.205 ab	3.823 ± 0.464a
Novosadska 32	3.518 ± 0.514a	0.750 ± 0.126a	0.420 ± 0.067 ab	11.440 ± 0.401 ab	6.876 ± 0.819a	5.292 ± 0.156a	2.960 ± 0.381a
Panonija	3.711 ± 0.605a	0.606 ± 0.013a	0.326 ± 0.033 ab	11.195 ± 0.376 ab	6.679 ± 0.565a	4.406 ± 0.466 ab	2.953 ± 0.540a
Novosadska crvena	3.999 ± 0.468a	0.814 ± 0.074a	0.366 ± 0.060 ab	9.606 ± 0.415b	6.399 ± 0.403a	4.393 ± 0.585 ab	2.932 ± 0.680a
Novosadska rana 2	3.318 ± 0.794a	0.679 ± 0.143a	0.355 ± 0.081 ab	10.633 ± 0.329 ab	6.133 ± 0.205a	3.923 ± 0.318 ab	2.109 ± 0.681a
Rumska crvenka	3.794 ± 0.853a	0.747 ± 0.102a	0.351 ± 0.052 ab	10.407 ± 0.189 ab	5.846 ± 0.480a	3.431 ± 0.174b	2.500 ± 0.506a
Bezostaya1	2.495 ± 0.264a	0.875 ± 0.065a	0.307 ± 0.038 ab	11.103 ± 0.187 ab	6.165 ± 0.824a	3.619 ± 0.298 ab	2.537 ± 0.671a
Bankut 1205	3.963 ± 0.569a	0.742 ± 0.131a	0.454 ± 0.016a	9.984 ± 0.386 ab	6.776 ± 0.748a	4.268 ± 0.161 ab	1.977 ± 0.817a
Crnozrna	3.252 ± 0.741a	0.818 ± 0.075a	0.419 ± 0.007 ab	12.209 ± 0.412 ab	6.700 ± 0.686a	4.358 ± 0.088 ab	2.194 ± 0.750a
Stara banatka	3.108 ± 0.392a	0.735 ± 0.068a	0.469 ± 0.009a	10.362 ± 0.846 ab	6.547 ± 0.471a	4.173 ± 0.562 ab	2.460 ± 0.561a
Velja pšenice	2.900 ± 0.269a	0.814 ± 0.063a	0.313 ± 0.057 ab	12.109 ± 0.557 ab	6.121 ± 0.170a	3.913 ± 0.429 ab	2.208 ± 0.675a
Garulja	3.577 ± 0.732a	0.706 ± 0.023a	0.352 ± 0.055 ab	12.010 ± 0.418 ab	6.079 ± 0.530a	4.855 ± 0.423a	2.920 ± 0.636a
NSD 3/92	3.400 ± 0.289a	0.670 ± 0.033a	0.215 ± 0.011b	11.589 ± 0.660 ab	7.051 ± 0.853a	4.106 ± 0.193 ab	3.539 ± 0.246a
F value	0.98 ns	1.39 ns	5.03***	2.50*	0.69 ns	2.35*	2.00*

3.4.2. Antioxidant activity

Antioxidant activity, expressed as mg Trolox equivalents per gram of dry matter (mg TE g^{-1} dm), varied among genotypes. Total antioxidant activity (TAA) ranged from 9.606 mg TE g^{-1} dm in Novosadska crvena to 12.576 mg TE g^{-1} dm in Mačvanka, with relatively high values also recorded in Crnozrna (12.209 mg TE g^{-1} dm), Velja pšenica (12.109 mg TE g^{-1} dm), Libellula (12.018 mg TE g^{-1} dm), and Garulja (12.010 mg TE g^{-1} dm). Genotype had a significant effect on TAA [$F(16, 34) = 2.50$, $p = 0.012$].

DPPH radical scavenging activity showed limited variation, ranging from 5.406 mg TE g^{-1} dm in Mačvanka to 7.051 mg TE g^{-1} dm in NSD 3/92, and did not differ significantly among genotypes [$F(16, 34) = 0.69$, $p = 0.784$]. ABTS activity ranged from 3.091 mg TE g^{-1} dm in Libellula to 5.292 mg TE g^{-1} dm in Novosadska 32, followed by Garulja (4.855 mg TE g^{-1} dm), and differed significantly among genotypes [$F(16, 34) = 2.35$, $p = 0.018$]. FRAP values ranged from 1.977 mg TE g^{-1} dm in Bankut 1205 to 4.392 mg TE g^{-1} dm in Dunav, with relatively high reducing power also observed in Novosadska 100 and Bačka. Genotype had a significant effect on FRAP [$F(16, 34) = 2.00$, $p = 0.045$].

Overall, Novosadska crvena, Bankut 1205, Rumska crvenka, and Panonija showed numerically high total phenol contents, although differences among genotypes were not significant. Stara banatka and Bankut 1205 had the highest flavonoid contents. In terms of antioxidant capacity, Mačvanka, Crnozrna, and Velja pšenica showed relatively high TAA values, whereas Novosadska 32 and Garulja had high ABTS activity, and Dunav showed the highest FRAP reducing power.

3.5. Correlation matrix between grain quality traits and susceptibility to *S. oryzae* in wheat genotypes

The correlation matrix was created to explore relationships between grain traits and susceptibility-related parameters of the genotypes (Fig. 4). Moisture content was positively correlated with protein content ($r = 0.34$, $p < 0.05$), and protein content showed a very strong positive correlation with dry gluten content ($r = 0.91$, $p < 0.001$), indicating that these grain-quality components tended to increase together. Among phenolic compounds, phenols and flavonoids were positively associated with each other ($r = 0.30$, $p < 0.05$). Focusing on susceptibility-related traits, the number of *S. oryzae* adults after four months showed a strong positive correlation with the percentage of consumed grains ($r = 0.85$, $p < 0.0001$). Both susceptibility indicators, progeny production and consumed grains (%), were positively correlated with grain moisture content ($r = 0.29$ to 0.34, $p < 0.05$), whereas they were negatively correlated with protein content ($r = -0.31$ to -0.36, $p < 0.05$) and dry gluten content ($r = -0.42$, $p < 0.01$). Among the phenolic compounds, phenols, flavonoids, and tannins all exhibited negative correlations with insect susceptibility parameters ($r = -0.27$ to -0.60, $p \leq 0.05$), indicating that higher concentrations of these metabolites were associated with reduced infestation and feeding. In contrast, FRAP values showed a strong positive correlation ($r = 0.53$ to 0.57, $p < 0.001$) with susceptibility, suggesting that, in this dataset, FRAP-captured antioxidant activity was associated with higher susceptibility-related values.

Grain susceptibility was also associated with grain color: higher lightness (L*), redness (a*), and yellowness (b*) values were associated with greater insect infestation and increased grain consumption ($r = 0.39$ to 0.61, $p < 0.05$). The color parameters were also negatively correlated with phenols ($r = -0.04$ to -0.31, $p \leq 0.05$ for a* only), tannins ($r = -0.26$ to -0.36, $p < 0.05$ for b* only), and flavonoids ($r = -0.36$ to -0.50, $p < 0.05$), suggesting an association between grain pigmentation, selected biochemical traits, and overall susceptibility to *S. oryzae*.

4. Discussion

The development of wheat varieties that are naturally (inherently) resistant to insect attack during grain storage represents one of the most promising alternatives to chemical insecticides in the management of stored-grain pests. In this context, comparing the resistance of different wheat genotypes to *S. oryzae* through bioassays is an essential first step in identifying resistant genotypes, and the present study provided clear and valuable insights in this area. The subsequent phenotyping of genotypes through a series of biochemical and physical tests allowed us to explore which specific grain traits are associated with resistance to *S. oryzae*, thereby helping to clarify traits associated with the observed differences in susceptibility and ultimately helping to reveal new potential sources of resistance to stored-product pests for use in breeding programs.

In this study, an interesting pattern emerged: most modern cultivars, aside from a few exceptions, generally showed higher susceptibility to *S. oryzae* infestation than older historical cultivars and landraces. Among the modern genotypes, Dunav, Novosadska 100, and Libellula were among the most susceptible, whereas all genotypes from the historical cultivars and landraces showed markedly stronger resistance, with Crnozrna, Bankut 1205, and Garulja being the least susceptible genotypes (based on progeny production and consumed grains (%)).

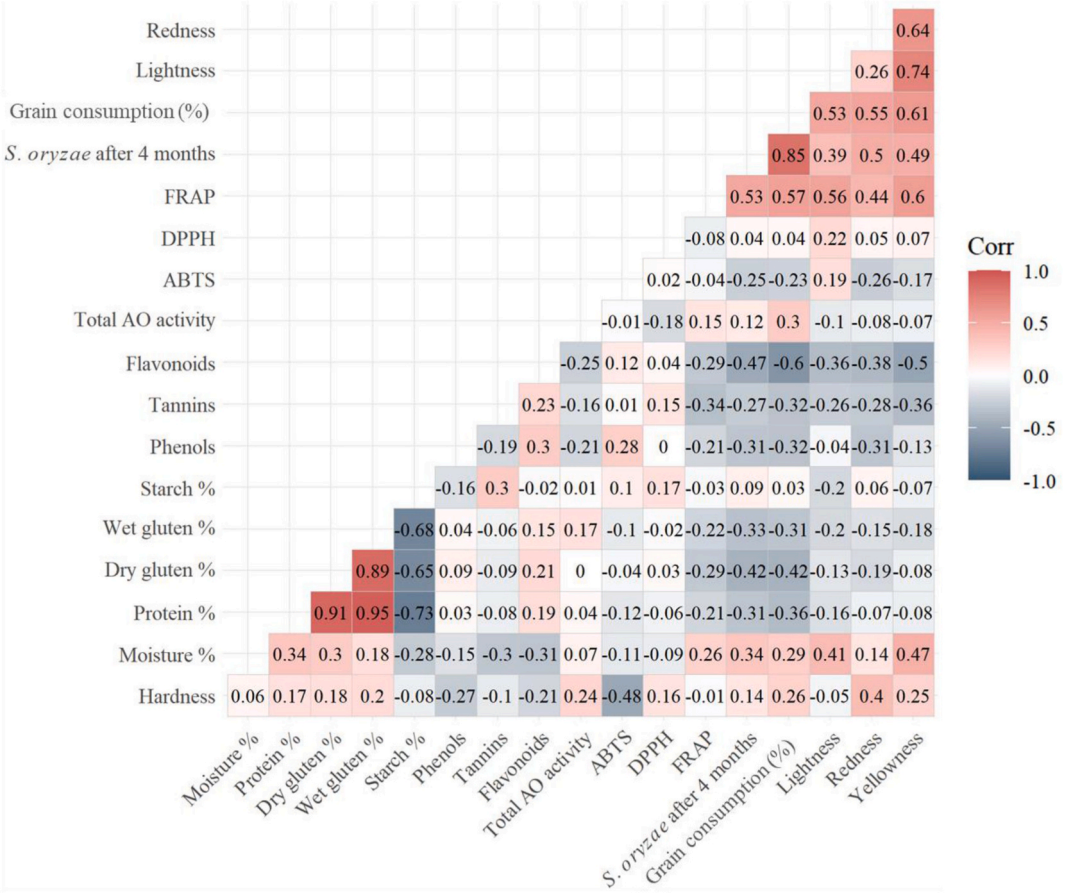

Fig. 4. Pearson correlation matrix among wheat grain quality traits, antioxidant capacity and susceptibility parameters to *S. oryzae* in wheat genotypes. Color intensity of the tiles indicates the strength and direction of the correlation (red = positive, blue = negative). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

This pattern was observed despite modern genotypes being recently developed and selected for contemporary agricultural requirements.

Our finding that historical cultivars and landraces were generally less susceptible to *S. oryzae* than most modern cultivars is consistent with other recent studies showing higher postharvest insect resistance in old cereal genotypes. D'Isita et al. (2024) reported lower susceptibility to *S. granarius* and *R. dominica* in several old durum wheat genotypes compared with modern varieties, while Sharma et al. (2024) found that traditional rice varieties suffered lower *S. oryzae* emergence and grain consumption than commercial cultivars. These findings suggest that modern breeding programs have often overlooked or unintentionally sacrificed the pest resistance traits that remain present in traditional landrace germplasm (Lazaridi et al., 2024; Newton et al., 2010). As a result, resistance during the storage phase has not been sufficiently prioritized, even though post-harvest protection is just as needed as protection against pests in the field, where resistance has received more attention.

Along with evaluation of the no-choice bioassay results, we analyzed biochemical and physical grain traits associated with these outcomes. The correlation heat map reveals several statistically significant associations that indicate how specific wheat grain characteristics are associated with *S. oryzae* population development and grain consumption during storage.

Regarding the nutritional components, higher protein and dry gluten percentages were associated with reduced susceptibility. Although the hardness measurement in this study showed only a weak correlation, gluten and protein still contribute to internal grain structure and density in ways that hardness meters do not fully capture (Kordan et al., 2023). High gluten content may reduce digestibility for insects, and elevated protein levels often correlate with the presence of defensive secondary metabolites, both of which may impair insect development (Arya et al., 2025).

These outcomes align well with previous research. Hashemi et al. (2025) demonstrated that wheat-grain traits strongly influence *S. granarius* performance, showing that higher protein content and greater grain strength reduce insect fecundity and population growth. Similarly, Arya et al. (2025) reported that higher protein content enhances resistance against *S. oryzae*, whereas higher carbohydrate levels facilitate faster insect development and increased susceptibility.

Higher moisture content was associated with increased susceptibility and weakened resistance to *S. oryzae*, an outcome that was expected and is consistent with findings from multiple other studies (Arya et al., 2025; Kordan et al., 2023; Mehta et al., 2021).

One of the important indicators of resistance was grain color, measured using a colorimeter. Color parameters showed significant and statistically significant correlations with insect performance. Lightness (L*) and yellowness (b*) were positively associated with both seed consumption and insect population size, while redness (a*) was also positively correlated with grain consumption. These relationships suggest that lighter and more yellow grains tended to be more susceptible to *S. oryzae*. This is an indirect effect, caused by phenolic compounds content that are responsible for coloration. Grain color parameters were negatively correlated with phenolic compounds. In particular, lower lightness values (darker grains) were associated with higher flavonoid concentrations, whereas total phenolic content showed weaker but significant negative correlations with color traits. Two of the most resistant genotypes, Velja pšenica and Crnozrna, were also among the darkest grains. In contrast, lighter and more yellow genotypes such as Libellula, Novosadska 100, and Dunav were among the most susceptible. However, this relationship was not universal; for example, Panonija, one of the more resistant modern cultivars, exhibited relatively low pigmentation. Typically, pigmented wheat varieties, particularly purple and blue wheat, are characterized by their high concentrations of anthocyanins, flavonoid-based phenolic pigments responsible for their distinct coloration. Because of their elevated anthocyanin levels, pigmented wheat genotypes generally exhibit much higher antioxidant activity compared to conventional yellow or white wheat. These biochemical differences may also influence how the grain interacts with storage pests (Ficco et al., 2014; Varga et al., 2013). A strong example of this resistance can be found in the study of D'Isita et al. (2023) which provides one of the clearest demonstrations that pigmented wheat can exhibit enhanced protection against stored-grain pests. In their work, a purple-pericarp durum wheat genotype (T1303) was compared with standard yellow-kernel varieties in bioassays with *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae), a major storage pest. Their results showed that the purple genotype was significantly more resistant. Adults feeding on T1303 consumed substantially less grain than those feeding on yellow wheat. In addition, the number of F_1 progeny produced per female was markedly lower on the purple wheat genotype. A similar association was observed in the present study. Significant negative correlations were found between these biochemical traits and both the number of newly emerged *S. oryzae* adults and the proportion of consumed grain. In general, darker grains tended to contain higher concentrations of phenols, flavonoids, and tannins, compounds previously associated with pest-deterrent properties, and genotypes with greater levels of these constituents supported fewer insects during storage. Together, these findings suggest that the biochemical profile associated with purple and blue pigmentation, particularly the elevated phenolic content, may be associated with natural resistance against storage pests.

A number of studies support the potential role of phenolic compounds, including tannins, phenols, and flavonoids, in enhancing grain resistance to *Sitophilus* species. Ramputh et al. (1999) demonstrated a strong negative correlation between phenolic content in sorghum and the emergence and feeding activity of *S. oryzae*, identifying phenolics as reliable biochemical markers of resistance. Similarly, Wongo (1998) showed that tannin-rich extracts significantly reduced feeding, delayed development, and suppressed progeny production in *S. oryzae*, indicating antifeedant and growth-inhibitory effects of tannins under experimental conditions. In wheat, Kordan et al. (2023) also found that higher concentrations of phenolic compounds were associated with reduced susceptibility to *S. granarius*. More recently, Arya et al. (2025) reported that variations in wheat phenolic acid composition were associated with differences in resistance to *S. oryzae*, supporting the potential role of phenolics as biochemical traits linked to reduced susceptibility. Comparable associations have also been reported in maize, where flavonoid accumulation was related to resistance to *Sitophilus* species, with higher flavonoid levels associated with reduced damage and insect performance (Lv et al., 2023).

Interestingly, in our study FRAP values were positively associated with *S. oryzae* susceptibility, which contrasts with most published work, where higher antioxidant and phenolic contents usually contribute to resistance. This suggests that, in our genotypes, FRAP values may reflect compounds that are not directly related to defensive activity, or that FRAP is linked with other susceptibility-enhancing traits.

These findings are consistent with previous reports indicating that traits associated with reduced susceptibility may be more prevalent in older wheat genotypes, supporting our observation that historical cultivars and landraces tended to exhibit greater resistance. Dinelli et al. (2011), for example, compared 16 old and 6 modern Italian common wheat varieties grown under uniform conditions and found that older cultivars possessed substantially higher levels of total polyphenols, flavonoids, and unique phenolic acid profiles than modern varieties. Similarly, Di Loreto et al. (2018) demonstrated that ancient durum wheat genotypes exhibit richer and more diverse phenolic profiles compared with modern durum cultivars. Regarding protein content, Ladhari et al. (2022) reported higher protein contents and a richer secondary-metabolite spectrum in old Tunisian durum landraces in comparison to modern cultivars.

5. Conclusions

This study shows that reduced susceptibility to *S. oryzae* in stored wheat was associated with a combination of biochemical and physical grain traits, and that these traits differed markedly between historical and modern genotypes. Across all bioassays, older cultivars and landraces generally showed lower *S. oryzae* emergence, reduced seed consumption, and overall lower susceptibility than most modern varieties. The grain phenotyping results indicate that darker, more pigmented genotypes characterized by higher concentrations of phenolic compounds, flavonoids, and tannins tended to be less susceptible. These metabolites, together with higher protein and gluten contents, showed negative correlations with insect development and feeding, suggesting that they may be useful indicators of reduced susceptibility in historical genotypes. The unexpected positive correlation between FRAP values and susceptibility suggests that antioxidant capacity alone does not reliably predict defensive potential and that the specific biochemical composition underlying antioxidant activity differs among genotypes.

When combined with earlier evidence that phenolics, flavonoids, and tannins can inhibit *Sitophilus* feeding and impair larval development, our findings suggest that these compounds may be involved in wheat resistance during storage. However, the relationships identified in this study are associative, and causal mechanisms require further validation through targeted experimental approaches. Since several of the traditional varieties that performed best in this study are no longer widely cultivated, their preservation is of critical importance. They constitute valuable reservoirs of resistance-related traits that have diminished or been lost in modern germplasm.

Future work should focus on identifying the genetic determinants underlying these biochemical traits, evaluating their heritability, and validating their functional role in resistance before incorporating them into breeding programs aimed at developing high-performing wheat cultivars with durable, intrinsic protection against stored-grain pests.

CRediT authorship contribution statement

Jullia Parsons: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Jordi Riudavets:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Milica Škorić:** Data curation, Formal analysis. **Sanja Mikić:** Resources, Supervision. **Radenka Kolarov:** Investigation. **Dejan Prvulović:** Formal analysis, Investigation, Methodology, Validation. **Snežana Tanasković:** Formal analysis, Investigation. **Sonja Gvozdenac:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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