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Evaluation of Short-Season Soybean (*Glycine max* (L.) Merr.) Breeding Lines for Tofu Production

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Abstract: Soybean breeding programs targeting tofu quality must evaluate their performance within zones of adaptation. A comprehensive study was carried out to examine soybean breeding lines from three maturity groups (MGs; MG0, MG00, and MG000) from 2018 to 2022. Several agronomic, chemical composition and tofu-related quality traits were evaluated, and the associations among traits were investigated. The results showed that genotypes in MG0 yielded higher and matured later, which confirmed that the selection of targeted genotypes for a specific maturity group was successful. Non-imbibed “stone seeds”, an important quality trait for tofu processors, were higher in MG000 lines. Tofu texture using both GDL and MgCl$_2$ coagulants was positively associated, indicating one coagulant might be enough for screening purposes. The MG by traits biplot showed very clear MG clustering for all genotypes tested from 2018 to 2022, signifying that the MG has a more pronounced effect on the investigated traits than the environmental effects seen in different years, regardless of the MG. Most tofu-related traits were higher and showed stronger associations in MG0 lines compared to the lines in earlier MGs, indicating a need for future effort in shorter season MGs. Overall, this study provided useful information for selecting soybean lines for tofu end-use application targeting specific MGs.

Keywords: soybean; tofu texture; quality screening; maturity group (MG)

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the major agricultural crops worldwide, with over 350 million metric tons of annual production [1]. Soybean planting offers a wide range of advantages to growers, including high yield, top protein quality with increasing industry demand [2], and an ideal place in crop rotation that minimizes nitrogen fertilizer requirement, improves soil quality, and promotes sustainable agriculture practices [3]. Non-GM (genetically modified) food-grade soybean for tofu markets is a niche sector with high premiums. The global consumption of soy-based foods including tofu has evolved significantly because of proven nutritional benefits, gluten-free and vegan labels, and affordability for consumers [4].

In addition to environmental factors (geographical location, soil type, temperature, and precipitation), genetic variation plays an important role in soybean protein variation [5–7]. Seed protein quantity and quality are the primary factors affecting tofu production. Some varieties are superior with respect to their soymilk and tofu-making performance [8–10]. To enhance the economic value and achieve success in the export market, it is essential to cultivate new soybean germplasm that embodies a comprehensive suite of traits deemed valuable from agronomic, disease resistance, and quality standpoints. This holistic approach ensures the development of robust and marketable crops that meet global standards for the tofu market. On the other hand, the soybean maturity group (MG) plays an important role in the adaptation of tofu soybean varieties to various environments. The MG classifies soybeans into various groups based on the duration between planting and harvest...
maturity and adaptation to, primarily, latitude. In short-season areas of Canada, three MGs are used in different regions, including MG0, the latest maturity, more suitable for parts of Southern Ontario; MG00, more adaptable to Quebec and the more northern parts of Ontario; and MG000, the earliest maturity, more suitable for the Canadian Prairies [11]. More research is needed to differentiate soybean breeding lines in various MGs for their tofu quality potential to allow for new high-quality varieties for each region.

There are several types of tofu, including firm, soft, pressed, and packed, with different recipes and final product texture, color, and mouthfeel, which are preferable for consumers in different countries, populations, or regions [12–15]. Tofu texture (firmness and consistency) is the main factor that differentiates tofu types and grades. A small-scale (SS) silken tofu test [16] was developed at the Ottawa Research and Development Centre (ORDC), AAFC, Canada, to address the need for a technique that is quick, efficient, reliable, which requires a low amount of sample, and can achieve analyzing large numbers of breeding lines in a timely manner. The method was tested against a well-accepted method (large-scale, LS) used for export samples [17]. The SS method ranked breeding lines similar to the LS method regarding their tofu texture. Further testing is required to evaluate the performance of the SS method across multiple years and soybean lines adapted to different MGs.

Soybean lines within different MGs exhibit varying behavior regarding their tofu production potential. Consequently, breeding efforts should be strategically directed toward specific MGs when selecting genetic material for parental crossing. To address the knowledge gap of tofu quality of soybean lines from multiple MGs and examine this hypothesis, a comprehensive study was carried out to evaluate soybean breeding lines from three MGs (MG0, MG00, and MG000) from 2018 to 2022 for their tofu performance. The goals were to: (1) analyze agronomic and chemical composition traits, (2) evaluate tofu-related quality traits, including stone seeds using the SS method, and (3) investigate the associations among all traits and potential patterns for a specific MG tofu performance.

2. Materials and Methods

2.1. Field Trials

Cultivar testing and phenotypic assessment were carried out, starting at the F4:6 generation. Genotypes were screened for agronomic and seed composition traits in non-replicated trials, from which selections were made. Selected genotypes were tested for tofu quality in replicated trials (two replications) in the Ottawa location (Ontario, Canada) at the F4:7 generation (known as screening trials). The soil type was North Gower imperfectly drained clay loam (Typic Endoaquolls), in the Orthic Humic Gleysol subgroup in Canadian soil classification. Check varieties were selected for each trial to represent agronomic adaptability to the target maturity group (MG). For example, AAC Edward is adapted to MG00 and MG000 in Western Canada, while OAC Lakeview represents commercial cultivars from MG0 in Ontario.

The trial design and layout of the screening trials were based on the number of total entries. The number of entries, including checks, may be inadequate to support the use of an ALPHA or lattice design (AGROBASE Generation 2, version 38.33.1, 2018), resulting in a randomized complete block (RCB) design being employed (Table S1). ALPHA and lattice designs are superior to RCB in that they divide each complete block into resolvable incomplete blocks where the average variance is generally smaller than what is seen in RCB. However, they can still be analyzed as RCB [18]. To minimize environmental variability, incomplete blocks of plots are kept grouped in a single range.

Field trials were planted between 16 May and 6 June using a Wintersteiger Oijord plot planter (Wintersteiger Inc., Saskatoon, SK, Canada) at the Central Experimental Farm of the Ottawa Research and Development Centre (45.3875° N, 75.7092° W), Ottawa, Ontario, Canada. Plots were 5 m long with four rows spaced 0.4 m apart, with 0.4 m inter-plot distance and a 1 m alley between ranges. The seeding density was 55.6 seeds/m². Pre-emergent herbicide was applied within 7 d of planting (Dual II Magnum, Syngenta, Guelph, ON, Canada; and Lorox L, NovaSource, Tessenderlo Kerley, Inc., Phoenix, AZ, USA), and
post-emergent herbicide was applied after the development of the first trifoliate and before the initiation of flowering (Basagran, BASF, Mississauga, ON, Canada; Excel Super, Bayer CropScience Inc., Calgary, AB, Canada; Pinnacle SG, FMC, Philadelphia, PA, USA; Assure 2, AMVAC, Newport Beach, CA, USA; and Venture L, Syngenta, Guelph, ON, Canada) and repeated if control was not adequate. No pre-harvest burndown was applied.

Complete plots were harvested between 23 September and 24 October using Hege 160 plot combines (Wintersteiger Inc., Saskatoon, SK, Canada) and stored in paper bags in an unheated building. To prevent spoiling, samples were dried to approximately 13% moisture content when needed using a natural gas Tytun Crop Dryer (Model TY-400, Tytun Ltd., Simcoe, ON, Canada).

From 2018 to 2022, a total of 396 soybean lines were tested for their tofu performance. These breeding lines were from three MGs, and two to five check varieties were in each trial. The experimental lines were not the same over all years. The complete details of breeding line names and their MG per year are presented in Table S2. The number of tested genotypes in each MG/year is presented in Table S1.

### 2.2. Agronomic Traits

Several agronomic traits were assessed in the field during the growing season. Maturity was assessed at R8 (where 95% of pods have reached their mature pod color) and represented as days since planting. Plant height was measured, in cm, at or near R8 as the main stem length from the ground to the node containing the top pod. Lodging was scored at the same time as plant height measurements, with fully upright plants being assigned a score of 1, and completely prostrate plants being assigned a score of 5, with plants leaning at 45° given a score of 3, and scores of 2 or 4 representing intermediaries.

Temperatures and precipitation were obtained from a meteorological station located at the Central Experimental Farm (data accessed on 15 March 2024 at [http://climate.weather.gc.ca/index_e.html](http://climate.weather.gc.ca/index_e.html)). The meteorological station was in a radius of 1 km from the experiment site. The mean values for temperature and precipitation from May to October in each year are presented in Figure 1.

![Figure 1. Mean values for temperature (columns) and total precipitation (blue lines) from May to October between 2018 and 2022. Error bars on columns are ±SE.](image)
Seed yield (kg/ha) was assessed in November or December following harvest using a laboratory balance (Sartorius Models LP6200S and LC6200; Sartorius Lab Instruments, Goettingen, Germany). Moisture was recorded at the time of weighing using a near-infrared whole grain analyzer (NIR) (FOSS Infratec 1241; FOSS North America, Eden Prairie, MN, USA), and the final yield was adjusted to a 13% moisture basis.

2.3. Seed Composition

Seed composition was assessed in November or December following harvest using an Infratec 1241 NIR and represented as % (w/w) on a dry matter basis. Seed weight was recorded as grams per 100 seeds using a laboratory balance (Shimadzu Model UW6200H; Shimadzu Corp., Kyoto, Japan; Mettle Toledo Model XS6001S; Mettler Toledo Canada, Mississauga, ON, Canada). Seed quality was scored visually, with a score of 1 being assigned to a seed with perfect appearance, and a score of 5 being assigned to a seed that was weathered, green, wrinkled, etched, or would fail to germinate. Scores 2, 3, and 4 were assigned based on the severity of the affliction and the ratio of good to bad seeds.

2.4. Assessment of Tofu-Related Quality

After assessing agronomic and seed composition, “poorly adapted” soybean lines with low yield, unsuitable maturity, severe lodging, etc., were discarded. Similarly, lines with a very dark hilum or discoloration of the seed coat were deemed to be unsuitable for tofu production and discarded. Samples were tested again for moisture, protein, and oil content using the Infratec 1241 NIR prior to tofu assessments.

Soymilk and tofu were prepared using the small-scale (SS) method [16] developed previously in the lab, with some modifications. The blended soymilk was passed through a juicer (Model 67601A; Hamilton Beach, Glen Allen, VA, USA) once, and the okara (retentate) was discarded. The coagulants used were 0.3% (w/v) glucono-delta-lactone (GDL) (D-(+)-Sigma–Aldrich Canada, Oakville, ON, Canada) and 0.15% (w/v) MgCl₂ (magnesium dichloride hexahydrate, GR ACS from EMD Chemicals Inc., Gibbstown, NJ, USA).

2.5. Statistical Analysis

Field trial setup and agronomic data analysis were carried out using AGROBASE Generation II (Version 38; Agronomix Software Inc., Winnipeg, MB, Canada) using built-in models for ALPHA, lattice, and RCB trial designs (Table S1). The genotype numbers were 77, 92, 53, 104, and 70 for 2018, 2019, 2020, 2021, and 2022, respectively. The agronomic and quality values presented in graphs or tables are the mean of all genotypes tested per each MG in each year ± SE. An Excel PivotTable (Microsoft 365, 2023) was used to calculate the mean values and standard errors.

To visualize and calculate the associations among soybean lines in each MG/year for agronomic, compositional, and tofu-related quality traits, a four-way data analysis statistical model was used [19]. The four independent parameters were the year, MG, repeat, and genotype. The genotype by traits biplots were generated for each year separately. The biplots were “trait-standardized” GT data (indicated by “Scaling = 1” and “Centering = 2” on the biplot, and the biplot origin shows the mean value for each trait) and “trait-focused” singular value partitioning (indicated by “SVP = 2”). A biplot analysis of MG by traits was also run, with all years included in the biplot, to visualize the traits’ association patterns in different MG.
3. Results and Discussion

3.1. Agronomy Traits

The mean values for temperature and total precipitation trends from May to October (planting to harvest period) from 2018 to 2022 are presented in Figure 1. The driest year during the pod-filling period (July, August, and September) was 2019, with an accumulated precipitation of 171.8 mm. The accumulated precipitation for the above-mentioned months exceeded 300 mm in 2018, 2020, and 2022. It has been shown that precipitation during the flowering and pod development period was the most influential environmental parameter for seed protein content in short-season soybeans [20], which increased at a rate of 0.17 g per kg seed for every 1 mm of precipitation. The mean temperature in the month of May (planting period) varied between 11.7 °C (in 2019) and 19.9 °C (in 2020). The mean temperature from June to September was above 15 °C for all years, and, overall, the hottest year was 2020.

The mean values of agronomy traits including seed yield, maturity, plant height, and lodging are shown in Figure 2. Overall, during 2018–2022, the MG0 lines yielded the highest, followed by the MG00 and the MG000 lines (grand yield average of 3336, 2801, and 2368 kg/ha, respectively). Yield is the main and the most important trait for all breeding programs. Our results are similar to the performance of ten soybean cultivars in different MGs (in Manitoba and Ontario, Canada), showing that for both locations, later maturity cultivars yielded significantly higher [11].

Days to maturity followed similar trends as yield (grand average of 112, 106, and 99 days for the MG0, MG00, and MG000 trials, respectively), which confirmed that the selection of targeted genotypes for a specific maturity group was successful. The average plant height ranged between 51 cm and 82 cm in various MGs and years. Plant height was higher in 2021 compared to 2019 for all three MGs; however, other years did not show any specific trend for MG0 and MG00. Most plants showed a good standability (the most minor lodging). Among all the lines tested, the soybean genotypes in the MG0 showed the worst lodging scores in 2018, 2020, and 2022 (1.3, 1.5, and 1.2 out of 5, respectively).

3.2. Seed Composition and Tofu-Related Traits

The soybean seed composition results, as well as the seed weight and seed quality scores, are summarized in Table 1. Seed weight ranged from 15.8 to 24.2 g, with a lower grand average value for MG000 than MG0 and MG00, which confirmed that soybean seeds in MG0 and MG00 trials had more plumpness (the seed weight was based on 100-seed weight). Seed quality was a subjective measurement used for selection. This quality trait was added to the calculation to explore whether there is any correlation between visually scored seed quality and the other measured tofu-related traits. The highest seed quality (perfect appearance) was assigned a score of 1, and the lowest received a score of 5 (weathering, green, wrinkled, etched, or unlikely to germinate). Overall, the genotypes in MG00 and MG000 performed better than the lines in MG0 in terms of seed quality scores. The genotypes in MG0 in 2020 and 2021 produced the best and the worst quality of seeds, respectively. Compositional traits (protein, oil, sugar, carbohydrates, sucrose, and raffinose/stachyose) were all in the expected range, with the genotypes in the MG0 trial having overall slightly higher protein content and lower oil content than MG00 and MG000 trials. The protein content range varied within each MG across all years (37.9–48.9%, 35.9–50.1%, and 37.4–47.1% for MG0, MG00, and MG000, respectively). Similar to our results, a study of soybean genotypes in both Manitoba and Ontario, Canada, did not show any specific trends regarding MG and protein content, although protein content was higher in Ontario than Manitoba; the variation was linked more to genetics and their interactions with environmental effects [11].
Figure 2. Mean values of yield (a), maturity (b), plant height (c), and lodging (d) of all tested genotypes in each year for a given MG. Error bars are ±SE.
Table 1. Mean values (±SE) of soybean seeds compositional analysis for all tested genotypes in each year for a given MG (Table S2).

<table>
<thead>
<tr>
<th>MG/Year</th>
<th>Seed Weight (g/100 Seeds)</th>
<th>Seed Quality (1–5 Score *)</th>
<th>Protein (% DM)</th>
<th>Oil (% DM)</th>
<th>Carbohydrates (% DM)</th>
<th>Sugar (% DM)</th>
<th>Sucrose (% DM)</th>
<th>Raffinose and Stachyose (% DM)</th>
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<tbody>
<tr>
<td>MG0</td>
<td></td>
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<tr>
<td>2018</td>
<td>20.3 ± 0.3</td>
<td>2.4 ± 0.1</td>
<td>43.6 ± 0.2</td>
<td>20.6 ± 0.1</td>
<td>17.4 ± 0.1</td>
<td>11.6 ± 0.1</td>
<td>5.9 ± 0.1</td>
<td>4.8 ± 0.0</td>
</tr>
<tr>
<td>2019</td>
<td>17.8 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>40.5 ± 0.3</td>
<td>20.9 ± 0.2</td>
<td>20.0 ± 0.1</td>
<td>12.7 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>4.3 ± 0.0</td>
</tr>
<tr>
<td>2020</td>
<td>21.9 ± 0.5</td>
<td>1.0 ± 0.0</td>
<td>44.7 ± 1.0</td>
<td>19.5 ± 0.5</td>
<td>19.1 ± 0.3</td>
<td>13.1 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>4.4 ± 0.1</td>
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<tr>
<td>2021</td>
<td>18.8 ± 0.4</td>
<td>3.2 ± 0.1</td>
<td>41.2 ± 0.4</td>
<td>21.9 ± 0.3</td>
<td>18.3 ± 0.1</td>
<td>11.4 ± 0.1</td>
<td>6.0 ± 0.1</td>
<td>4.8 ± 0.0</td>
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<tr>
<td>2022</td>
<td>22.3 ± 0.3</td>
<td>2.4 ± 0.1</td>
<td>44.4 ± 0.2</td>
<td>19.5 ± 0.1</td>
<td>18.0 ± 0.1</td>
<td>11.6 ± 0.1</td>
<td>6.5 ± 0.1</td>
<td>4.4 ± 0.0</td>
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<td>MG00</td>
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<tr>
<td>2018</td>
<td>24.2 ± 0.4</td>
<td>2.3 ± 0.1</td>
<td>44.1 ± 0.2</td>
<td>20.7 ± 0.1</td>
<td>16.8 ± 0.1</td>
<td>11.1 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>4.8 ± 0.0</td>
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<tr>
<td>2019</td>
<td>16.6 ± 0.2</td>
<td>2.1 ± 0.0</td>
<td>39.0 ± 0.2</td>
<td>21.9 ± 0.1</td>
<td>20.3 ± 0.1</td>
<td>12.5 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>4.1 ± 0.0</td>
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<tr>
<td>2020</td>
<td>21.1 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>44.1 ± 0.5</td>
<td>20.1 ± 0.2</td>
<td>17.1 ± 0.2</td>
<td>10.6 ± 0.1</td>
<td>5.9 ± 0.1</td>
<td>4.4 ± 0.0</td>
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<tr>
<td>2021</td>
<td>17.0 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>42.9 ± 0.3</td>
<td>20.9 ± 0.2</td>
<td>19.0 ± 0.1</td>
<td>11.5 ± 0.1</td>
<td>6.2 ± 0.1</td>
<td>4.4 ± 0.0</td>
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<tr>
<td>2022</td>
<td>20.6 ± 0.4</td>
<td>1.4 ± 0.1</td>
<td>42.1 ± 0.3</td>
<td>20.9 ± 0.2</td>
<td>18.9 ± 0.1</td>
<td>12.0 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>4.3 ± 0.0</td>
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<tr>
<td>MG000</td>
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<tr>
<td>2019</td>
<td>15.8 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>38.8 ± 0.3</td>
<td>21.9 ± 0.2</td>
<td>20.4 ± 0.2</td>
<td>12.5 ± 0.1</td>
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<td>4.1 ± 0.0</td>
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<tr>
<td>2021</td>
<td>16.7 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>43.1 ± 0.4</td>
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<td>11.5 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>4.4 ± 0.0</td>
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* 1 best, perfect seed; 5 low quality, no germination anticipated.
A study of applying heat stresses at the initial seed-filling stage (20 °C vs. 30 °C) under a controlled environment revealed that higher temperatures would alter the plant metabolic pathway to produce more lipids and fewer proteins [21]. Our study conducted on a much larger group of soybean genotypes showed a different trend; the month of July (equal to the initial seed-filling stage) was slightly hotter in 2018 and 2020 than in 2019, 2021, and 2022 (Figure 1). However, the mean values of protein content for genotypes in 2018, 2020, and 2022 were higher in the MG0 trials. In the MG00 trials, the mean protein values were higher in 2018 and 2020. The genotypes in the MG000 trials showed lower protein content in the hotter July of 2019 compared to 2021. The combination effect of temperature and precipitation might explain the differences in protein accumulation in different MGs and years. The main subunits of seed storage protein (including 7S and 11S) accumulate gradually from the early to late stage of seed filling [22], which affects both the content and functionality of protein in soybeans. Interestingly, the position of seeds within a plant (apical or basal nodes) also influences the accumulation of storage protein subunits [23].

The quality traits of the tofu production using the SS method are presented in Figure 3. The quality trait referred to as “stone seeds” is the percentage of seeds that remained hard or partially imbibed after overnight soaking (18 h at 18 °C). Among all years and MGs, the genotypes tested in the MG000 trials showed the highest stone seeds for both years (8.5% and 8.9% in 2019 and 2021, respectively). In the MG00 trials, 2021 had the highest percentage of stone seeds (7.1%) compared to other years. In both 2019 and 2021, the precipitation patterns were different compared to other years; the pod-filling period (July–September) was dry, followed by a very wet harvest month (158.7 mm and 140.4 mm precipitation in October for 2019 and 2021, respectively). This might influence the early maturity genotypes in MG00 and MG000 to produce harder grains with a poor imbibition ability. Several studies have shown that the permeability of soybean seeds is governed by the seed coat, which is influenced by the environment [24–28]. To the best of our knowledge, this trait and its effect on tofu production have not been investigated in literature. Stone seeds are an important quality trait for tofu processors, affecting the tofu yield and, thus, profitability. The Ottawa Soybean Breeding program closely monitors and selects genotypes regarding this quality trait. The results of this study could facilitate the better selection of genotypes for the MG000 region, targeting lower stone seeds.

The results of soymilk dry matter and Brix indicated that these two traits followed similar trends, which was expected. The SS method adjusts the soybean weight used in tofu production to a constant water-to-dry matter protein ratio, resulting in a typical 10 to 12% Brix. For all MGs in all years, the GDL coagulant produced slightly firmer tofu compared to the MgCl$_2$ coagulant. A similar trend was observed in soybean genotypes tested using the same SS method in 2013–2015 [16]. Tofu production using MgCl$_2$ leads to a faster aggregation rate and a less dense network structure [29]. This type of tofu is desirable for certain markets. Tofu firmness depends on the protein quality (11S/7S ratio) and the coagulant’s type and concentration [5,7,10,30,31]. Gels formed from soybean protein rich in the 11S group showed a greater extent of macro-phase separation and a coarser network, while soybean protein gels rich in the 7S group produced a more homogeneous aspherical aggregate structure [32]. Harder tofu can be achieved by soybeans having a higher ratio of 11S/7S protein, while varieties with a lower ratio require a higher coagulant concentration to achieve maximum hardness [33]. In particular, a significant correlation was found between the A3 subunit of the glycinin group (11S) and the firmness of a type of tofu similar to the silken tofu used in our study but with a different preparation recipe [5,34]. When testing soybeans with a range of subunit profiles, using the SS method, it was concluded that soybeans lacking α’ A4 subunits produced the firmest tofu, and as more subunits were missing in the protein, the tofu texture weakened [16]. At the optimum level, a coagulant would produce tofu with the firmest texture and more uniform cells/tofu network [35]. In our study, among lines from different MGs, the MG0 lines showed higher firmness for both coagulants (grand average of 4.8 N and 3.8 N for GDL and MgCl$_2$ coagulants, respectively).
Figure 3. Mean values of tofu-related traits, including stones (%), Brix (%), dry matter (% of soy milk), and tofu texture firmness (N force), using GDL or MgCl₂ coagulants. Error bars are ±SE.

3.3. GGEbiplot Analysis

The results previously reported for the SS method [16] focused on the method’s efficacy in predicting tofu hardness compared with the LS method. In our study, however, a comprehensive analysis was performed to visualize the association of all agronomic, compositional, and tofu-related traits using a much larger sample of soybean breeding lines from multiple years and various MGs.

The associations between traits in each year were visualized using GGEbiplot (version 8.2, 2023), and are presented in Figures 4–8. The biplots sufficiently predicted the associations between traits; the sum of PC1 and PC2 (biplot fitness) was 39.7%, 46.5%, 67.8%, 54.2%, and 55.7% for genotypes tested in 2018, 2019, 2020, 2021, and 2022, respectively. Traits with longer vectors in the biplots were more discriminating among genotypes. Also, the angle between the trait vectors approximates the strength of the correlation between them, with an angle smaller than 90° showing a positive correlation, an angle greater than 90° showing a negative correlation, and a right angle (90°) showing no correlation. A biplot shows multiple relationships in the same figure, while Table S3 presents the numerical values for the individual correlations.
Figure 4. GGEBiplot visualization of trait association for all genotypes tested in 2018. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and MgCl$_2$ (TEXTURE GDL and TEXTURE MGCL2, respectively). The circles represent genotypes in MG0 (blue circles) and MG00 (green circles). The genotype names are presented in Table S2.

The agronomic traits showed strong associations with several compositional and tofu-related quality traits during the five-year study (Figures 4–8). Since the primary goal of any breeding effort is yield improvement, any other traits targeted for specific improvement should not negatively impact yield. In our study, the yield was correlated positively with maturity, which showed the strongest associations in 2020, 2021, and 2022 ($r$ values of $+0.868$, $+0.813$, and $+0.842$, respectively). These results confirmed that late maturity genotypes yielded higher. Figures 7 and 8 show that seed quality was also positively correlated with yield and maturity (long biplot vectors), showing higher quality soybean seeds in MG0. Yield showed different patterns of associations with tofu-related traits in different years, which makes the selection for tofu-related traits more complex. Kurasch et al. [36] reported no significant association between yield and tofu traits. Interestingly, protein and yield showed different association patterns in different years. There was a significant negative correlation in 2019 and 2021 ($-0.245$ and $-0.432$, respectively), while in 2022, the correlation was positive ($+0.437$), and in 2018 and 2020, the correlation was...
insignificant. Our results confirm the contradicting reports of the association between yield and protein in the literature, ranging from negative [37] to insignificant [38]. Soybean yield and seed protein content are complex traits. Many associated quantitative trait loci (QTL) have been identified, facilitating the development of high-protein and high-yielding soybean genotypes [39–42]. Since protein is the main factor affecting tofu yield and texture, our results show that combining high-yield and firmer tofu traits is possible. Plant height and lodging association were different in different years, with hotter and wetter years (2018, 2020, and 2022) having a positive correlation (+0.694, +0.575, and +0.283, respectively), while drier years and years with less precipitation (2019 and 2021) did not show a significant correlation. However, lodging was not a major concern in any given year, with plants showing an acceptable standability. Seed size (measured by 100-seed weight) was associated positively with yield in 2020, 2021, and 2022 but was insignificant in 2018 and 2019. Larger seed sizes are preferable for tofu manufacturers, and having a positive association with yield would be beneficial for growers. Seed size association with tofu texture was not consistent in different years. Our findings confirmed the inconsistency in the literature, with some reporting positive, or negative, or insignificant associations between seed size and tofu-making performance [36]. This might be due to the complex genetic interaction of seed size and other traits.

Figure 5. GGEBiplot visualization of trait association for all genotypes tested in 2019. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and MgCl$_2$ (TEXTURE GDL and TEXTURE MGCL2, respectively). The circles represent genotypes in MG0 (blue circles), MG00 (green circles), and MG000 (brown circles). The genotype names are presented in Table S2.
Figure 6. GGEBiplot visualization of trait association for all genotypes tested in 2020. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and MgCl$_2$ (TEXTURE GDL and TEXTURE MGCL2, respectively). The circles represent genotypes in MG0 (blue circles) and MG00 (green circles). The genotype names are presented in Table S2.

The oil and protein content showed a strong and significantly ($p < 0.05$) negative association for all years; from 2018 to 2022, the $r$ values were $-0.647$, $-0.813$, $-0.958$, $-0.936$, and $-0.876$, respectively (Table S3). Our results agree with a well-documented negative association between oil and protein in soybeans [37,38,43,44]. In all years, the contents of sugar, carbohydrates, and sucrose were significantly associated with each other, with sucrose and carbohydrates showing the highest $r$ value (between 0.757 and 0.933). The association between tofu texture using GDL or MgCl$_2$ as the coagulant was significant for all years except 2022 ($r$ values between 0.327 and 0.694). A previous study reported no significant coagulant-specific clustering using the SS method, using a much smaller sample size tested in three years [16]. Soymilk dry matter did not show a consistent pattern with tofu texture with either coagulant, being positive or negative in some years and insignificant in 2018 with MgCl$_2$. Similar results of no direct effect of soymilk on tofu firmness have also been reported [36]. Protein content showed a very strong and negative association
with soymilk dry matter and soymilk Brix; this was expected since, in the SS method, the amount of soybean was adjusted to a constant ratio of water-to-dry matter protein, which means higher protein lines required less soybean for the soymilk production, resulting in less soymilk dry matter. Protein content was significantly and positively associated with tofu texture using the GDL coagulant but not the MgCl$_2$ coagulant. This could be explained by the fact that protein (content and composition) and coagulant type both influence the texture of tofu. It has been reported in the literature [5,33–35] that, in soybeans with lower 11S, a higher amount of coagulant is needed to achieve tofu with similar hardness.

Figure 7. GGEBiplot visualization of trait association for all genotypes tested in 2021. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and MgCl$_2$ (TEXTURE GDL and TEXTURE MGCL2, respectively). The circles represent genotypes in MG0 (blue circles), MG00 (green circles), and MG000 (brown circles). The genotype names are presented in Table S2.
Figure 8. GGEBiplot visualization of trait association for all genotypes tested in 2022. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and MgCl$_2$ (TEXTURE GDL and TEXTURE MGCL2, respectively). The circles represent genotypes in MG0 (blue circles) and MG00 (green circles). The genotype names are presented in Table S2.

To understand the overall behavior of genotypes from different MGs, a biplot analysis of MG by traits was run, with all years being included in the biplot (Figure 9). A very clear MG clustering was observed for all genotypes tested from 2018 to 2022. This indicates that the genotypes within each MG have a more pronounced effect on the investigated traits than the environmental effects seen in different years. Yield was more positively associated with tofu texture GDL than tofu texture MgCl$_2$ in the MG0 trial. Most tofu-related traits were higher and showed stronger associations in MG0. The genotypes in MG00 were strongly and negatively associated with plant height and sugar content. The genotypes in MG000 showed higher associations with carbohydrate and oil content and higher stone seeds, Brix, and dry matter. Historically, the ORDC breeding program had a long-term emphasis in the later maturity group on food quality traits, while the earlier maturity material emphasized yield and early maturity. Only more recently have crosses and selection been targeting food type traits across all maturities. The higher tofu quality
seen in the later maturity group reflects the historical differences in breeding objectives between maturity groups. Many QTLs underlying soybean agronomic traits, including maturity, have been mapped, and can be applied for future breeding of soybean lines for a specific MG. Soybean adaptation to different latitudes/daylengths is controlled by maturity genes $E_1$ to $E_{11}$ and their interactions with QTLs for Time of Flowering ($Tof_5$, $Tof_{11}$, $Tof_{12}$, and $Tof_{16}$) \cite{45}. The gene $E_8$ was specifically linked to early or late maturity in soybeans \cite{46}. It has been reported that the QTLs on chromosomes 6 and 19 correspond to the maturity genes $E_1$ and $E_3$, respectively \cite{47}.

Figure 9. GGEBiplot visualization of trait by MG in all years and for all the genotypes tested. Traits comprised yield, maturity, plant height, lodging, seed quality, stone seeds (STONES), seed weight, protein, oil, carbohydrates (CARBO), sugar, sucrose, raffinose and stachyose (RAFFSTACH), soymilk Brix, soymilk dry matter, and tofu texture firmness using GDL and $\text{MgCl}_2$ (TEXTURE GDL and TEXTURE $\text{MgCl}_2$, respectively). $\text{Mg}0$, $\text{Mg}00$, and $\text{Mg}000$ refer to the different maturity groups.

4. Conclusions

In this multiple-year research, agronomic, chemical composition, and tofu-related quality traits were evaluated to investigate the associations between soybean traits targeted for specific maturity groups (MGs) regarding their tofu performance. In all three MGs,
yield correlated positively with maturity. As expected, a strong negative correlation was found between protein and oil. The MG by trait biplot revealed that most tofu-related traits are positively associated with MG0 genotypes. Both coagulants, GDL and MgCl₂, showed significant correlations for the tofu texture trait. Measuring the non-imbibed “stone seeds”, an important quality trait for tofu processors, demonstrated that the MG000 lines are more prone to this unfavorable trait and need to be monitored more closely.

Overall, this five-year research project provided the opportunity to develop and offer 12 new soybean lines to the industry. The lines were developed for various MGs in western and eastern Canada. Also, the high hard seed phenomenon observed in 2019 and 2021 in MG0 and MG000 resulted in selecting against lines prone to developing hard seeds. The ORDC breeding program seems to be stratified from later maturity with higher tofu quality to early maturity with less tofu-making quality, which clearly reflects the introgression of tofu-related quality traits from late-maturity donors and the historical emphasis on food quality traits in MG0.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/seeds3030028/s1, Table S1: Design of field trials and the number of tested genotypes used for tofu testing from 2018 to 2022; Table S2: The complete list of tested soybean genotypes in the various MG trials from 2018 to 2022; Table S3: The correlation matrix among measured traits from 2018 to 2022. Statistically significant ($p < 0.05$) $r$ values are highlighted in green.

Author Contributions: Conceptualization, E.R.C. and M.H.; methodology, E.R.C. and M.H.; validation, S.L. and M.H.; formal analysis, M.H.; investigation, S.L.; resources, S.L., M.H. and E.R.C.; writing—original draft preparation, M.H.; writing—review and editing, S.L., M.H. and E.R.C.; supervision, E.R.C. and M.H.; project administration, E.R.C. and M.H.; funding acquisition, E.R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agriculture and Agri-Food Canada and the Canadian Field Crop Research Alliance (CFCRA), J-002097.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This project was funded by Agriculture and Agri-Food Canada and the Canadian Field Crops Research Alliance, through the Canadian Agricultural Partnership program. The authors would like to acknowledge the assistance of all staff and students involved in this project.

Conflicts of Interest: The authors declare no conflicts of interest.

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