

Thousand seeds weight in industrial hemp genotypes - Comparative analysis

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Abstract

The study evaluated thousand seeds weight (WTS) in several genotypes of industrial hemp. The field researches were done within ARDS Lovrin. Seven genotypes of industrial hemp were used in the present study: Hg601, Hg602, Hg603, Hg618, Hg622, Hg625, and Hg628. The crop was carried out in non-irrigated conditions, in repetitions for each genotype. The seeds were harvested at physiological maturity. Thousand seed weight was determined by the classical method, by weighing modules of one thousand seeds each. A normal distribution of WTS values was recorded, with $r = 0.986$. In relation to the mean value (WTS_m = 17.691 g), there were positive differences in the Hg603 genotype ($p < 0.01$, **), and in the HG602 genotype ($p < 0.05$, *). Negative differences were recorded for the Hg618 genotype ($p < 0.05$, o). In the case of the median value, a positive difference was recorded for the Hg603 genotype ($p < 0.05$, *), and a negative difference for the Hg618 genotype ($p < 0.05$, o). The cluster analysis grouped the genotypes based on similarity into two distinct clusters (Coph.corr. = 0.775). The WTS parameter is important for industrial hemp for sowing, and for crops quality. At the same time, it is also important for the valorization of seeds in the food industry, seeds with high WTS values show a high consistency, with a high useful fraction (kernels) for different functional products.

Keywords: biometric parameters, industrial hemp genotypes, quality indice, seeds, WTS

Introduction

Thousand seed weight (TSW) is a genetically determined seed quality parameter that varies within certain limits under the influence of environmental and technological factors [4], [9], [17].

The size of the seeds was analyzed in relation to the germination and the quality of the seedlings in different plant species [1], [10]. In relation to several size categories of tobacco seeds, the authors recorded a differentiated response of the plants, with advantages for plants resulting from larger seeds. Thousand seeds weight is an important factor for seed germination, seedling generation, seedling growth and yield [1]. The authors studied these aspects in medicinal plants, and concluded the importance of seed size on seedling growth.

The influence of seed weight was studied on plant growth yield [2]. Based on the study, the authors recorded that larger seeds determined more vigorous plants, with higher growth yield, with better physiological indices compared to the situation when smaller seeds were used. The TSW parameter is important in relation to the yield of crops, the valorization of seeds, for sowing, or valorization on the market, industrialization, etc. [6], [17]. Seed weight was considered an important attribute for the harvest index and soybean yield [5].

Thousand seeds weight is an important parameter with influence on the costs of plant production and market promotion of genotypes [15]. The authors analyzed these aspects in eggplant, and recorded the importance of the parameter for the description of the genetic base and the assisted selection of genotypes. Seed weight in plants is a specific trait, an important morphological parameter, with differentiated advantages for seed quality, species dissemination [18].

Seed weight was studied in several barley genotypes to analyze genotypic correlations between traits

[7]. The author communicated that the WTS parameter was one of the most stable. Biometric parameters of seeds show importance for the strategies of plant breeding programs [11]. The authors analyzed the importance of increasing seed weight in rapeseed to increase yield, without affecting oil production. Studies on flax (*Linum usitatissimum* L.) were carried out to understand the involvement of genomic factors in the definition of TSW [17].

Determining the TSW parameter by the classical method (counting seeds, weighing) is quite cumbersome and time-consuming and human resource-consuming [6]. Alternative methods, based on reduced seed samples, or imaging analysis have been tested [6]. For the determination of TSW, the random selection of seeds is an important factor in obtaining objective results and the packaging of seeds for the purpose of distribution and valorization on the market [13]. The use of counters for automatic seed counting is useful, and the method has been tested in different species with variable seed sizes [13]. Methods based on imaging analysis have been tested and promoted for phenotyping seeds and determining the weight of one thousand seeds [19]. The authors obtained regression models based on which it was possible to estimate the weight of a thousand seeds with high precision.

Starting from the importance of seed weight reported in different plant species, the present study analyzed thousand seeds weight and compared several industrial hemp genotypes.

Material and Method

The study analyzed the variation of the WTS parameter (thousand seed weights) in industrial hemp genotypes.

Field experiments were organized within ARDS Lovrin, with different genotypes of industrial hemp. Seven genotypes were considered in the study, noted: Hg601, Hg602, Hg603, Hg618, Hg622, Hg625, and Hg628. The hemp genotypes were cultivated in four repetitions, non-irrigated crop system.

At physiological maturity, 99 BBCH code [12] plants were harvested, on experimental variants. The seeds were taken, and they were selected for the elimination of foreign bodies (fragments of inflorescences, leaves, etc.).

For each genotype, thousand seeds weight was determined, per repetition. For this, seed subsamples were taken from the seed sample from each genotype. One thousand seeds from each subsample were counted, figure 1. The seeds were weighed with a laboratory balance (± 0.005 g). Data series were recorded for each industrial hemp genotype.

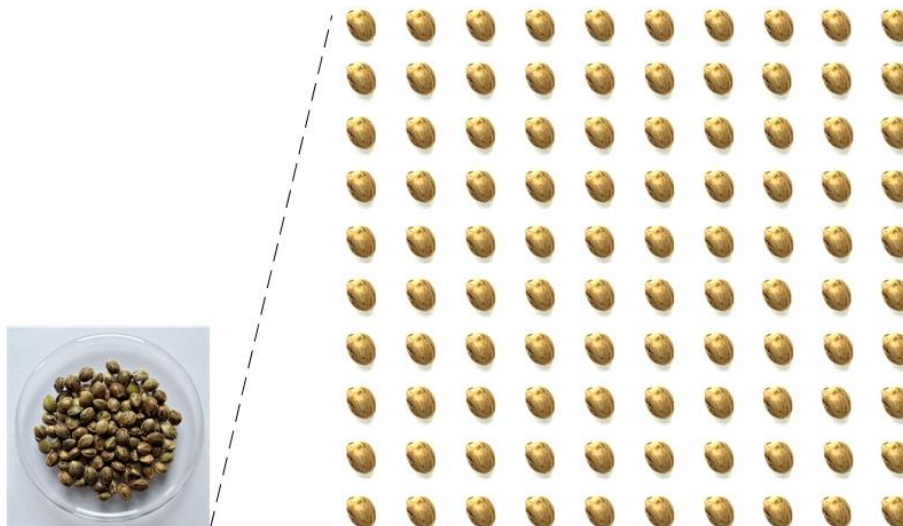


Figure 1. Work module in counting a thousand seeds (original image, by Florin Sala)

The experimental data were analyzed in EXCEL (the mathematical and statistical calculation module) and with the PAST software [8]. Appropriate tests and analysis tools were used, in relation to the purpose of

the study. To interpret the differences, safety thresholds were used ($p < 0.05$; $p < 0.01$; $p < 0.001$).

Results and Discussion

Thousand seed weight (WTS, g) parameter for industrial hemp genotypes showed average values of WTS = 17.60 g for the Hg601 genotype, WTS = 19.00 g for the Hg602 genotype, WTS = 19.40 g for the Hg603 genotype, WTS = 16.16 g for genotype Hg618, WTS = 17.00 g for genotype Hg622, WTS = 18.06 g for genotype Hg625, and WTS = 16.62 g for WTS628 genotype, respectively. A normal distribution of WTS values was recorded, with $r = 0.986$, figure 2.

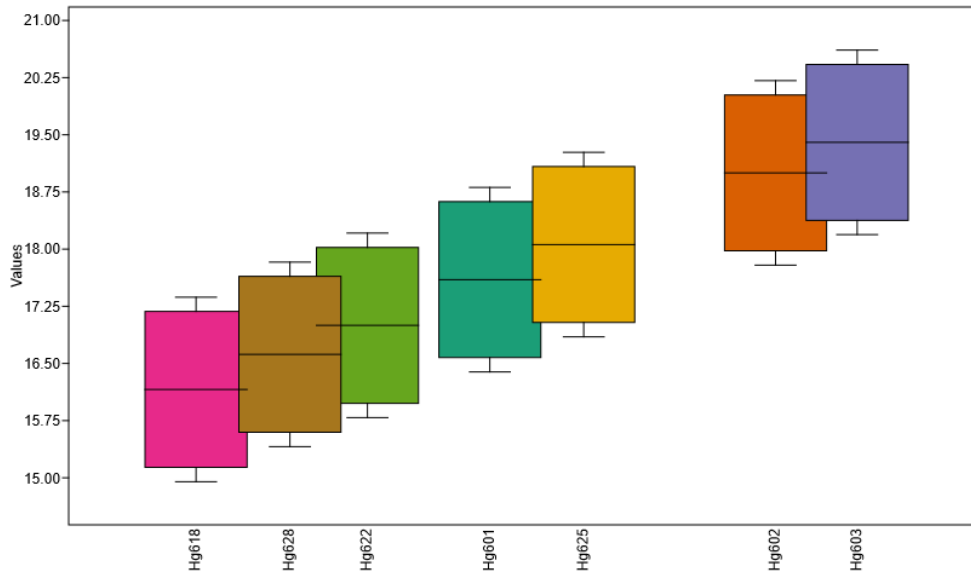


Figure 2. Distribution of values for WTS in industrial hemp seeds

Hemp genotypes were comparatively analyzed based on the recorded WTS values. The ANOVA test was applied (t-Test, and Wilcoxon Test). The results were the values presented in table 1. Compared to the average value calculated at the level of the tested genotypes ($WTS_m = 17.691$ g), the differences presented different situations, for each genotype.

Positive differences were recorded in the case of the Hg603 genotype, at the level of $p < 0.01$ (**), and in the case of the HG602 genotype, at the level of $p < 0.05$ (*). The negative difference was recorded in the case of the Hg618 genotype, at the level of $p < 0.05$ (o). In the case of the other genotypes, the differences between the WTS values in relation to the average value did not show statistical certainty, table 1. The graphic representation of the WTS values in relation to the mean value is presented in figure 2.

In relation to the median value (17.600 g), the genotype Hg603 presented positive differences at the level of $p < 0.05$ (*), and the genotype Hg618 presented negative differences at the level of $p < 0.05$ (o). In the case of the other genotypes, the differences did not show statistical certainty.

The recorded results are presented in detail in table 1, with statistical analysis parameters and recorded values for each genotype. The significance of the differences is also presented, for each genotype, in relation to the thresholds of statistical significance.

Table 1. Anova Test results

Statistical parameters	Industrial hemp genotype						
	Hg601	Hg602	Hg603	Hg618	Hg622	Hg625	Hg628
t-Test							
Given mean:	17.600	19.000	19.400	16.160	17.000	18.060	16.620
Sample mean:	17.691	17.691	17.691	17.691	17.691	17.691	17.691
95% conf. interval:	(16.575 18.808)	(16.575 18.808)	(16.575 18.808)	(16.575 18.808)	(16.575 18.808)	(16.575 18.808)	(16.575 18.808)

Difference:	0.091	1.309	1.709	1.531	0.691	0.369	1.071
95% conf. interval:	(-1.0252 1.2081)	(0.1919 2.4252)	(0.5919 2.8252)	(0.41476 2.6481)	(-0.42524 1.8081)	(-0.7481 1.4852)	(-0.045243 2.1881)
t :	0.2003	-2.8674	-3.7439	3.3558	1.5151	-0.8076	2.3478
p (same mean):	0.8478	0.0285	0.0096	0.0153	0.1805	0.4501	0.0572
Significance of difference	ns	*	**	o	ns	ns	ns
Wilcoxon Test							
Given median:	17.600	19.000	19.400	16.160	17.000	18.060	16.620
Sample median:	17.600	17.600	17.600	17.600	17.600	17.600	17.600
W :	11	20	21	21	17	15	19
Normal appr. z :	0.1048	1.9917	2.2014	2.2014	1.3628	0.9435	1.7821
p (same median):	0.9165	0.0464	0.0277	0.0277	0.1730	0.3455	0.0747
p (exact):	1.0000	0.0625	0.0313	0.0313	0.2188	0.4375	0.0938
Significance of difference	ns	ns	*	o	ns	ns	ns

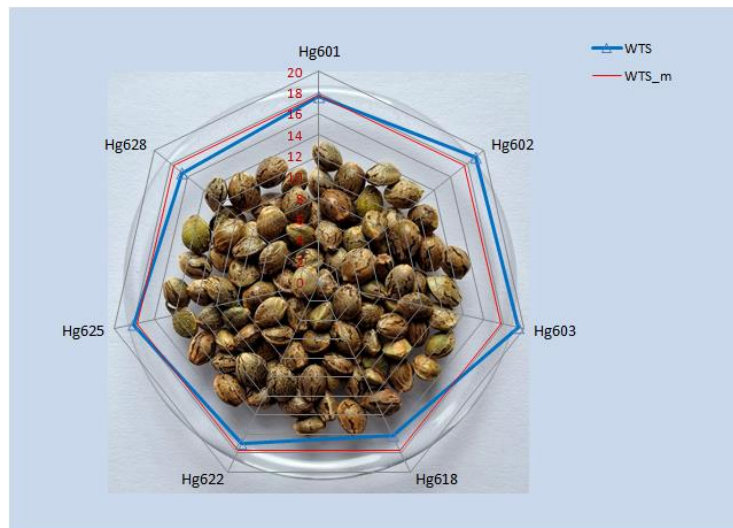


Figure 2. WTS values distribution for industrial hemp genotypes (blue line) in relation mean value (red line)

The cluster analysis led to the grouping dendrogram of the genotypes based on the mean values of the WTS parameter (Coph.corr. = 0.775), figure 3. The genotypes Hg602 and Hg603, with values above the mean, were grouped in the C1 cluster. The other five genotypes were grouped in the C2 cluster, within two sub-clusters, based on similarity. The calculated SDI values, which showed the level of similarity between the genotypes, are presented in table 2. In the case of genotypes Hg602 and Hg603, with high values of the WTS parameter, the level of dissimilarity was SDI = 0.400. The highest level of similarity was recorded between the genotypes Hg622 and Hg628, with SDI = 0.380.

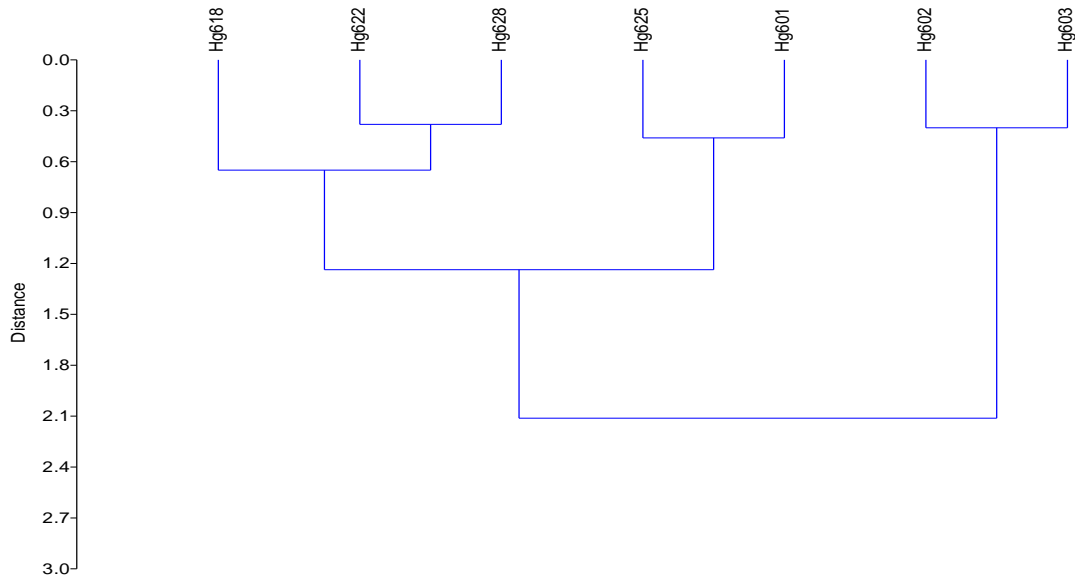


Figure 3. Dendrogram grouping industrial hemp genotypes, based on WTS values

Table 2. SDI values for industrial hemp genotypes, in relation to the WTS parameter

	Hg601	Hg602	Hg603	Hg618	Hg622	Hg625	Hg628
Hg601		1.400	1.800	1.440	0.600	0.460	0.980
Hg602	1.400		0.400	2.840	2.000	0.940	2.380
Hg603	1.800	0.400		3.240	2.400	1.340	2.780
Hg618	1.440	2.840	3.240		0.840	1.900	0.460
Hg622	0.600	2.000	2.400	0.840		1.060	0.380
Hg625	0.460	0.940	1.340	1.900	1.060		1.440
Hg628	0.980	2.380	2.780	0.460	0.380	1.440	

Scientific literature has highlighted the importance of seed parameters, among which TSW, which generated advantages for obtaining plants and agricultural crops, for yield, for plant breeding programs and promoting genotypes on the market [2], [6], [15], [17]. Industrial hemp mainly ensures the production of fiber, and secondarily the production of seeds, which are of interest to the food industry, as a source of protein, minerals, nutritional principles, functional foods [3], [14], [16]. The results of this study compared seven industrial hemp genotypes, and classified the genotypes in relation to the value of the WTS parameter. The study contributes to data bases development regarding the quality of industrial hemp seeds, with applications for agricultural practice, and seeds valorization.

Conclusions

Seven genotypes of industrial hemp were analyzed under the aspect of the seed quality index, and they expressed different values for the WTS parameter (thousand seed weight). The recorded data series showed normal distribution, with $r = 0.986$. In crop conditions in non-irrigated system, the genotypes Hg602 and Hg 603 showed values of the WTS parameter above the mean value of the experiment, in statistical safety conditions (HG602 genotype, at the level of $p < 0.05$, *; Hg603 genotype, at the level of $p < 0.01$, **). The Hg618 genotype presented negative differences, at the level of $p < 0.05$ (o).

The Cluster analysis grouped the studied genotypes based on similarity in relation to the WTS parameter. A high level of similarity was recorded between the genotypes Hg622 and Hg628, with $SDI = 0.380$. In the case of the genotypes Hg602 and Hg603, with high values of the WTS parameter, the level of dimilarity was $SDI = 0.400$. The recorded results show the importance of breeding programs for industrial hemp, in order to progress towards high values of seed quality parameters.

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References

- [1] Afshari, H., Eftekhari, M., Faraji, M., Ebadi, A.G., Ghanbarimalidareh, A. (2011), *Studying the effect of 1000 grain weight on the sprouting of different species of Salvia L. grown in Iran*. Journal of Medicinal Plants Research, 5(16): 3991-3993.
- [2] Bečka, D., Bečková, L., Satranský, M., Pazderů, K. (2024), *Effect of seed weight and biostimulant seed treatment on establishment, growth and yield parameters of winter oilseed rape*. Plant, Soil & Environment, 70(5): 296-304.
- [3] Burton, R.A., Andres, M., Cole, M., Vowley, J.M., Augustin, M.A. (2022), *Industrial hemp seed: from the field to value-added food ingredients*. Journal of Cannabis Research, 4: 45.
- [4] Chen, K., Łyskowski, A., Jaremko, Ł., Jaremko, M. (2021), *Genetic and molecular factors determining grain weight in rice*. Frontiers in Plant Science, 12: 605799.
- [5] Duc, N.T., Ramlal, A., Rajendran, A., Raju, D., Lal, S.K., Kumar, S., Sahoo, R.N., Chinnusamy, V. (2023), *Image-based phenotyping of seed architectural traits and prediction of seed weight using machine learning models in soybean*. Frontiers in Plant Science, 14: 1206357.
- [6] Felix, F.C., Mocelim, F.L., Torres, S.B., Kratz, D., Ribeiro, R., Nogueira, A.C. (2021), *Thousand-seed weight determination in forest species by image analysis*. Journal of Seed Science, 43: e202143040.
- [7] Hadjichristodoulou, A. (1990), *Stability of 1000-grain weight and its relation with other traits of in dry areas*. Euphytica, 51: 11-17.
- [8] Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001), *PAST: Paleontological Statistics software package for education and data analysis*. Palaeontologia Electronica, 4(1): 1-9.
- [9] Hladni, N., Jocić, S., Miklič, V., Miladinović, D., Zorić, M. (2016), *Interrelationship between 1000 seed weight with other quantitative traits in confectionary sunflower*. Ekin Journal of Crop Breeding and Genetics, 2(1): 51-56.
- [10] Kasperbauer, M.J., Sutton, T.G. (1977), *Influence of seed weight on germination, growth, and development of tobacco*. Agronomy Journal, 69(6): 1000-1002.
- [11] Labra, M.H., Struik, P.C., Evers, J.B., Calderini, D.F. (2017), *Plasticity of seed weight compensates reductions in seed number of oilseed rape in response to shading at flowering*. European Journal of Agronomy, 84: 113-124.
- [12] Meier, U. (2001), *Growth stages of mono- and dicotyledonous plants e BBCH monograph*. Federal Biological Research Centre for Agriculture and Forestry, 158 pp.
- [13] Milivojević, M., Vujinović, J., Branković-Radojčić, D., Vukadinović, R., Petrović, T. (2022), *Monitoring of seed counter in 1000 seed weight testing*. Journal on Processing and Energy in Agriculture, 26(1): 34-37.
- [14] Montero, L., Ballesteros-Vivas, D., Gonzalez-Barrios, A.F., Sánchez-Camargo, A.D.P. (2023), *Hemp seeds: Nutritional value, associated bioactivities and the potential food applications in the Colombian context*. Frontiers in Nutrition, 9: 1039180.
- [15] Qian, Z., Ji, Y., Li, R., Lanteri, S., Chen, H., Li, L., Jia, Z., Cui, Y. (2022), *Identifying quantitative trait loci for thousand grain weight in eggplant by genome re-sequencing analysis*. Frontiers in Genetics, 13: 841198.
- [16] Rizzo, G., Storz, M.A., Calapai, G. (2023), *The role of hemp (Cannabis sativa L.) as a functional food in vegetarian nutrition*. Foods, 12(18): 3505.
- [17] Saroha, A., Gomashe, S.S., Kaur, V., Pal, D., Ujjainwal, S., Aravind, J., Singh, M., Rajkumar, S., Singh, K., Kumar, A., Wankhede, D.P. (2023), *Genetic dissection of thousand-seed weight in linseed (Linum usitatissimum L.) using multi-locus genome-wide association study*. Frontiers in Plant Science, 14: 1166728.
- [18] Törő-Szilygyártó, V., Balogh, N., Henn, T., McIntosh-Buday, A., Sonkoly, J., Takács, A., Kovacsics-Vári, G., Cando, P.D., Molnár, V.A., Matus, G., Teleki, B., Süveges, K., Lukács, B.A., Lovas-Kiss, Á., Tóthmérész, B., Tóth, E., Tóth, K., Török, P. (2023), *New thousand-seed weight dataset for plant species of Central Europe*. Data in Brief, 48: 109081.
- [19] Zhang, H., Ji, J., Ma, H., Guo, H., Liu, N., Cui, H. (2023), *Wheat seed phenotype detection device and its application*. Agriculture, 13: 706.