J. Agr. Sci. Tech. (2022) Vol. 24(5): 1205-1215

Canonical Correlations between Nanomecanical Properties and Some Agronomic Traits in Sugar Beet

A. Teimoori Yane-Sari[1], A. Hematiyan[1], D. Davoodi[2*], F. Roozbeh[3]

ABSTRACT

The purpose of this research was to study the relationships between surface nanomechanical properties and agronomic traits in different sugar beet varieties (*Beta vulgaris* spp.). Agronomic traits were related to the indicators of seed germination stage and resistance to rhizomania; and in correspondence, a group of nanomechanical traits of inner testa of seeds were examined using an atomic force microscope. The results of parametric and non-parametric correlation analysis between agronomic and nanomechanical traits showed that the single bud wet weight had a significant negative and positive relationship with, respectively, lower surface friction and adhesion of the inner testa. Similarly, thousand shell weight had a negative correlation with upper surface elasticity, and also seed vigor had a positive correlation with upper surface friction. Spearman's rho correlation coefficient showed that resistance to rhizomania also had a significant positive correlation with the upper surface adhesion of the inner testa. Three canonical variables between the two groups of physiological and nanomechanical traits were significant (r1= 0.972 and DF= 66; r2= 0.924 and DF= 50; r3= 0.839 and DF= 36). These traits have a kind of cause-and-effect relationship and, therefore, have the potential to be used for breeding programs and plant systematic studies.

Keywords: Atomic Force Microscopy, *Beta vulgaris* spp., Nanomarker selection, Resistance to rhizomania,

INTRODUCTION

Plant breeding has a long history and dates back to about eleven thousand years ago. At the beginning of this period, farmers chose edible plants with desirable nutritional properties and used them for cultivation in later years (Kaiser *et al*., 2020). The selection process has been inherited from farmers to plant breeders and has evolved to the present day as they use the latest genetic and molecular techniques for selection. In parallel with the evolution and development of new methods of plant breeding, the role of indirect selection, such as Marker-Assisted Selection (MAS), has become more prominent than direct selection. The main condition for success in indirect selection is that the indicator or marker has a significant correlation with the trait intended by the breeder or the goal of the breeding program (Jiang, 2013). The higher correlation, the more guaranteed it is to achieve the goal of the breeding program. Indirect selection can be a very good solution when traits are difficult or expensive to record (Fellahi *et al*., 2018). In general, knowledge of the relationships between traits is essential for plant breeding, as indirect selection can

[1] Department of Nanobiotechnology, Faculty of New Sciences and Technologies, Tehran University, Tehran, Islamic Republic of Iran.
[2] Department of Nanotechnology, Agricultural Biotechnology Research Institute of Iran, Agricultural Research, Education and Extension Organization, Shahid Fahmideh BLVD, Karaj, Islamic Republic of Iran.
[3] Plant Gene Bank, Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization, Shahid Fahmideh BLVD, Karaj, Islamic Republic of Iran.
[*]Corresponding author; e-mail: ddavoodi1339@gmail.com

accelerate the achievement of promising genotypes (Trevisani *et al*., 2017). In the meantime, plant physiology and, consequently, the relationship between physiological traits and agronomic traits are essential for the effective use of new techniques in plant breeding (Bekis, 2020). Most sugar beet breeding and improvement programs have initiated assisted breeding efforts, primarily as a first step to discover marker associations with traits (McGrath, 2010). Marker Assisted Selection (MAS) plays an important role in sugar beet breeding and industry, so, private companies produce hybrid sugar beet seed for commercial planting. They have extensive cultivar development programs, including MAS capabilities, and much of the yield gains worldwide over the past several decades are directly due to their efforts (McGrath and Panella, 2018). Norouzi *et al*. (2015) studied the efficiency of some molecular markers related to rhizomania resistance genes for marker selection in sugar beet and, based on the results, concluded that the selected markers can be used for marker-assisted selection to increase the efficiency of selection and reduce the time and cost of sugar beet breeding programs.

It is now well accepted that the cell functions are essentially determined by its structure and certain mechanical properties (Kuznetsova *et al*., 2007). For example, the beet pericarp can restrict the water and oxygen uptake of an enclosed seed (Hermann *et al*., 2007). These mechanical properties are in turn controlled by genetic factors, so, the structure of the pericarp, the size of the basal pore and its degree of tissue filling are genetically determined by the maternal genotype (Chomontowski and Podlaski, 2020). Atomic force microscopy has been used extensively to study sugar beet pectin (Fishman *et al*., 2010, Gromer *et al*., 2009, Kirby *et al*., 2006), but no report was found for a similar purpose in our study.

In some studies, the microscopic structures of the plant have been used as criteria for distinguishing cultivars, because the microstructure of plant tissue is its fundamental material feature and determines its other properties –chemical, physical, and biological- (Konstankiewicz, 2011). Ghosh *et al*. (2009) studied seed microstructures in 26 species of four genera, namely, *Acacia*, *Albizia*, *Cassia*, and *Dalbergia* using scanning electron microscopy; and identified specific patterns of organization for each species. In this way, they were able to isolate these species using scanning electron micrographs. Guha *et al*. (2001) used a scanning electron microscopy and atomic force microscopic contact mode to study the ultrastructure properties of different mash plantations. They concluded that the atomic force microscope was more desirable because it did not require special preparation for the sample compared to the electron microscope. On the other hand, both cotyledon and the seed coat of different lines have unique supra-structural details, and AFM images were able to distinguish these lines from each other. Rashid *et al*. (2018), by studying leaf epidermal anatomy of tribe *Trifolieae* L. using electron microscopy, concluded that combination of leaf epidermal characters in correlation with other traits has potential for taxonomic resolution at species level.

Nanotechnology is one of the newest technologies that has emerged in the last two decades in the field of human life and has introduced tools and methods for studying biological and non-biological materials at the nanoscale (1×10^{-9} m) (Ciambelli *et al*., 2020). This technology has overshadowed all sciences and industries, including plant sciences, and despite the few studies that have been done with nanotechnology tools and methods in this area, there is a great potential for exploiting the technology in relevant basic and applied research. Atomic Force Microscopy (AFM) is a versatile technique of surface characterization, providing accurate information about the topography and other wide variety of magnitudes at nano scale (Benítez *et al*., 2019). This technique has been introduced alongside common microscopic techniques to study the fine details of plant tissues at the nanoscale. Farahi *et al*. (2017) showed the correlation between chemical treatments and changes in surface nanomechanical properties by studying the plasticity, elasticity, and adhesion energy of poplar plant cell walls using atomic force microscopy. Kozlova *et al*. (2019) went a step further and proposed an AFM approach for assessing the nanomechanical properties of primary cell walls in the inner tissues of growing plant organs. Based on the proposed method, they examined the Vibratome-derived longitudinal and transverse sections of maize root by AFM in a liquid cell to track the changes of cell wall stiffness and elasticity accompanying elongation growth.

Plant nanomechanics is a subset of plant biomechanics and advances in research in this field have a great impact on plant biomechanics in general because the properties and characteristics of the nanoscale inevitably affect macroscopic appearance and performance (Burgert and Keplinger, 2013). In recent years, various topics of structure-property and structure-performance relationships have been the subject of a large volume of plant mechanical characterization studies (Burgert and Keplinger, 2013). As mentioned above, the nanomechanical properties of cells and plant tissues determine their function.

With an area under cultivation of about 200,000 hectares (FAO, 2010), Iran is one of the main producers of sugar beet (*Beta vulgaris* L.) in the Middle East. Both sugar beet production and sugar industries have a significant role in Iran's agriculture and agro-industries regarding technological, economic, and social development of rural communities (Bazrgar *et al*., 2011).

This study was conducted to investigate the correlations between nanomechanical properties of plant tissues and agronomic traits as well as their possible candidacy as nanomarkers for use in sugar beet (*Beta vulgaris* L.) plant breeding programs.

MATERIALS AND METHODS

To find the relationships between some important agronomic traits and nanomechanical properties of seed surfaces in sugar beet, an experiment was conducted in a Completely Randomized Design (CRD) with three replications. Plant experiment was carried out in the Sugar Beet Seed Institute (SBSI) and microscopic studies were performed in the Nanotechnology Research Department of the Agricultural Biotechnology Research Institute of Iran (ABRII).

Plant Materials

Seeds of ten sugar beet genotypes that differed in important physiological traits, such as germination rate and resistance to rhizomania (the most serious disease in sugar beet worldwide (Neher and Gallian, 2014)), were selected. The seeds of these genotypes were obtained from the Sugar Beet Seed Institute (SBSI) of Iran and a summary of their specifications is given in Table 1.

Table 1. Selected monogerm cultivars of sugar beet and some of their properties in addition to resistance to rhizomania.These lines also differed in their germination characteristics.

No	Sugar beet line	Specifications
1	31888-90	Pollinator S1 line- Tolerant to rhizomania
2	Brigita	Commercial line- Resistant
3	31928-90	Pollinator S1 line- Resistant
4	Jolgeh	Commercial line - Susceptible
5	SBSI 005	Resistant
6	SBSI 006	Resistant
7	31923-90	Pollinator S1 line- Tolerant
8	31924-90	Pollinator S1 line- Tolerant
9	31889-90	Pollinator S1 line- Tolerant
10	Gadook	Commercial line - Susceptible

Seeds

Seeds of the selected cultivars were first screened and standardized according to the International Seed Testing Association (ISTA, 1985) protocols.

Traits Measurement

Agronomic traits included Thousand Kernel Weight (TKW), seed viability [Germination Percentage (G%)], Germination Velocity (GV), Mean Germination Time (MGT)), Root and Shoot Length (RL and SL), Wet and Dry Weight of single seedling (WW and DW), Thousand Shell Weight (TSW), Thousand Embryo Weight (TEW), and Vigor (V). Also, topography, adhesion, friction, and elasticity of the lower and upper surfaces of the inner testa of the seed embryo were examined and measured using an atomic force microscope.

TKW

After standardization, from the seed mass of each line, three replications of each 1,000 seeds were separated using a divider device, and after accurate counting, the weight of the repetitions was determined (TKW). Seed viability test was performed according to the recommended method of ISTA (1985). In each replication, the germination percentage, germination velocity, and mean germination time (50% germination time) were calculated using the following equations, respectively:

$$Germination\ percentage\ (G\%) = \frac{Number\ of\ germinated\ seeds\ per\ replication}{Total\ number\ of\ seeds\ per\ replication} \times 100$$

$$Germination\ Velocity\ (GV) = 100 \times \frac{\sum Ni}{\sum Ni\ Di}$$

Where, Ni: Number of seeds germinated on the ith day, Di: Number of Days counted from the day of sowing to the collection of the datum (i).

$$Mean\ Germination\ Time\ (MGT) = \frac{\sum Ni\ Di}{S}$$

Where, Ni: Number of seeds germinated on the ith day, Di: Number of Days counted from the day of sowing to the collection of the datum, and S is the Sum of the germinated seeds at the end of the experiment.

By measuring the weight of seed shells and embryo of one gram of seeds, thousand seed shell weight and thousand embryo weight were calculated.

Seed Vigor

In order to measure the vigor, 25 seeds from each sample were randomly selected and planted at a distance of one cm in boxes that contained soil up to a height of ten centimeters in the greenhouse. Each replication was placed randomly in a separate box. Daily counting of buds was performed for fourteen days and then dry weight was measured for each replication (ISTA, 1995). The following equation was used to calculate vigor:

$$Vigor = Germination\% \times Dry\ weight$$

Atomic Force Microscopy Imaging and Measurements

Seed inner testa (Figure 1) (Hermann et al., 2007) was used to study and measure the nanomechanical properties. Some tissues, such as the outer testa, were so rough that they could not be scanned under an AFM.

Sample Preparation

A number of seeds from each line were soaked in water for 24-48 hours and the operculum was removed using a scalpel under stereomicroscope. After separating and removing the operculum and outer testa, which was brown (see in Figure 1-D), the inner testa, which was light orange in color, was separated and transferred to a slide. Parts as small as 2×2 mm of the inner testa were attached to the microscope sample holder with a double-sided tape adhesive. The upper and lower (lower and upper) surfaces of the specimens were scanned with contact and non-contact modes of the AFM.

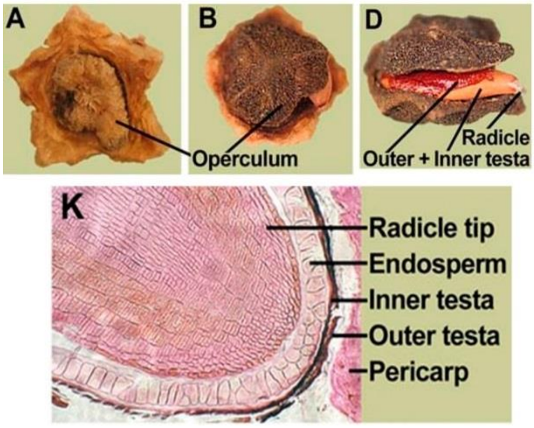

Figure 1. Sugar beet seed (A, B and D) with graphic representation of radicle area components (K). Compared to other tissues, the inner testa had a good smoothness for scanning with AFM.

Friction (Lower and Upper Surfaces of the Inner Testa)

DC mode (Contact mode) was used to calculate the friction because in this mode, the AFM light base can measure the friction between the tip and the sample. Images from the two-way scan (round trip) of the probe tip on the surface of the sample were prepared, and by dividing the two images, the amount of friction was determined based on the voltage between the probe tip and the surface (in volts or millivolts).

Adhesion (Lower and Upper Surfaces of the Inner Testa)

AC mode (non-contact mode) was used to measure adhesion force. Topographic images were taken from each line in three replications and the degree of adhesion was obtained from the spectroscopic diagram.

Elasticity (Lower and Upper Surfaces of the Inner Testa)

Young's modulus is a measure of stiffness. To measure the Young's modulus with the AFM device, the method of creating supersonic vibration in the cantilever connected to the probe was used. Based on the change in the oscillation spectrum of the AFM cantilever, the Young's modulus or elasticity of the surface was examined.

Data Analysis

As mentioned earlier, this experiment was performed in a completely randomized design with three replications and statistical analyses including F-test, ANOVA, mean comparison, paired-sample t-test and correlation coefficients were performed with SPSS (IBM SPSS Statistics Version 22) software. In the correlation test for nanoscopic traits with resistance to rhizomania, the non-parametric Spearman rank correlation coefficient was used (Spearman, 1904). Finally, the canonical correlation analysis was performed in order to determine the relationships between the two groups of physiological germination variables and nanomechanical variables of the inner seed testa.

RESULTS AND DISCUSSION

Although some reports suggest that it is more or less impossible to study the internal tissues of the plant due to the high roughness that causes damage to the AFM probe (Kozlova *et al*. 2019), it has been shown here that some plant tissues, such as inner testa without embedding and cutting, can be examined with an atomic force microscope.

By performing F-test and ensuring that the data were normal, a significant difference between the cultivars was assessed by the Analysis Of Variance (ANOVA) test (Table 2) and showed that the difference between the genotypes was statistically significant (P<

0.05 or 0.01) for most physiological and all nanoscopic traits. Only three traits of germination velocity, single bud dry weight, and mean germination time did not differ significantly between genotypes. In Table 3, the paired-samples t-test and correlation analysis was performed for nanoscopic trait data to show significant differences and relationships between these traits. The test results showed that there was a very significant positive correlation between the upper and lower surfaces of the inner testa in terms of friction, adhesion, and elasticity traits, and both surfaces followed a similar pattern. However, it was only in the trait of friction that the upper and lower surfaces differed significantly. Of course, there are strong structural relationships between these nanoscopic traits and, as Alazemi *et al*. (2016) showed, there is a direct correlation between the two traits of adhesion and friction.

The results of parametric and non-parametric correlation analysis between agronomic and nanoscopic traits showed that the single bud wet weight had a significant negative and positive relationship with lower surface friction and adhesion of the inner testa, respectively (Tables 4 and 5). Similarly, thousand shell weight had a negative correlation with upper surface elasticity, and vigor had a positive correlation with upper surface friction. Spearman's rho correlation coefficient showed that resistance to rhizomania also had a significant positive correlation with the upper surface adhesion of the inner testa (Table 5). To find the most

Table 2. The ANOVA for physiological and nanoscopic traits of germination process in ten sugar beet genotypes.

Physiological traits[a]	df	Mean square	F	Nanoscopic traits[a]	df	Mean square	F
TKW	9	4.426	8.750**	LSF	9	1841213.260	1810.589**
GV	9	0.445	2.083ns	USF	9	2964648.770	1677.218**
MGT	9	0.013	1.851ns	LSA	9	90304.033	224.135**
G%	9	241.126	7.037**	USA	9	228524.700	149.568**
RL	9	4.868	7.474**	LSE	9	0.952	14.090**
SL	9	2.276	10.605**	USE	9	2.052	11.897**
WW	9	0.001	5.794**				
DW	9	0.000	0.732ns				
TEW	9	0.619	2.983*				
TSW	9	3.675	14.548**				
V	9	1591.984	7.790**				

* and **: Significant at 0.05 and 0.01 levels, respectively (2-tailed); ns: Not significant.
[a] TKW= Thousand Kernel Weight; GV= Germination Velocity; MGT= Mean Germination Time; G%= Germination Percentage; RL= Root Length; SL= Shoot Length; WW= Single bud Wet Weight; DW= Single bud Dry Weight; TEW= Thousand Embryo Weight; TSW= Thousand Shell Weight; V= Vigor; LSF= Lower Surface Friction; USF= Upper Surface Friction; LSA= Lower Surface Adhesion; USA= Upper Surface Adhesion; LSE= Lower Surface Elasticity, USE= Upper Surface Elasticity.

Table 3. Paired samples t-test for nanoscopic traits of paired upper and lower surfaces of sugar beet seed inner testa.

		Paired Samples				
		Differences			Correlations	
		Mean	Std error mean	t	R^2	Sig
Pair 1	LSF - USF	-194.9470000	81.6167090	-2.389*	0.890878	0.000**
Pair 2	LSA - USA	-2.0000000	39.8189870	-0.050ns	0.583941	0.001**
Pair 3	LSE - USE	-0.1170000	0.1223338	-0.956ns	0.638106	0.000**

* and **: Significant at 0.05 and 0.01 level respectively (2-tailed); ns- not significant.

Table 4. Simple parametric correlation analysis between seed germination physiological traits and nanoscopic traits of testa in ten genotypes of sugar beet (Pearson's correlation coefficient).

	LSF	USF	LSA	USA	LSE	USE
TKW	-0.307	-0.180	0.209	-0.207	-0.179	-0.265
GV	-0.273	-0.273	0.237	0.339	0.260	0.336
MGT	0.249	0.246	-0.215	-0.315	-0.243	-0.311
G%	0.099	0.250	0.123	-0.272	-0.049	0.063
RL	0.237	0.187	-0.247	-0.204	-0.094	-0.326
SL	0.015	0.010	-0.013	-0.033	0.356	-0.030
WW	-0.500**	-0.278	0.453*	0.295	0.124	0.095
DW	-0.093	-0.147	0.137	0.046	0.197	0.207
TEW	0.075	0.058	0.080	-0.189	-0.071	-0.023
TSW	-0.305	-0.142	0.084	-0.115	-0.262	-0.381*
V	0.327	0.515**	-0.286	-0.254	-0.077	0.023

* and **: Significant at 0.05 and 0.01 level respectively (2-tailed).

Table 5. Simple non-parametric correlation analysis between rhizomania resistance and nanoscopic traits of testa in ten genotypes of sugar beet (Spearman's rho correlation coefficient).

	LSF	USF	LSA	USA	LSE	USE
Resistance to rhizomania	0.098	-0.213	-0.154	0.368*	0.167	0.343

* Significant at 0.05 level (2-tailed).

correlated linear combination of physiological and nanoscopic variables, canonical correlation analysis was performed (Table 6). Three of the six canonical variables between the two groups of physiological and nanoscopic traits were significant (r1= 0.972, DF= 66 and α= %1; r2= 0.924, DF= 50 and α= %1; r3= 0.839, DF= 36 and α= %5). In the first canonical variable, three traits of G%, SL and GV from the group of physiological traits (Table 7) as well as nanoscopic traits USF, USA and LSA (Table 8) were important and effective. Similarly, for the second canonical variables, three traits i.e. TSW, GV and MGT, and three traits from the group of physiological traits i.e. GV, MGT and TSW, and corresponding to them for the third canonical variables, three traits LSF, LSA and USF and three traits LSF, LSA and USA from the group of nanoscopic traits had the largest contribution, respectively. The results indicate that the relationship between adhesion and friction traits of the upper and lower surfaces of the inner testa, which incidentally have a high correlation with each other, with agronomic traits is much stronger than elasticity.

In the field of biology, mechanical forces are rarely recognized as major regulators of development processes. But the ability of plants to sense and transmit mechanical signals suggests that complex interactions between mechanics and chemistry are possible during plant development (Dumais 2007). Undoubtedly, the nanomechanical properties of plant tissue surfaces depend on proteins, fats, and carbohydrates, which in turn affect seed germination and vigor (Moraes *et al*., 2006). Search in scientific literature shows the relationship between the mechanical properties of cells and tissues with plant growth phenomena. Kozlova *et al*. (2019) studied the mechanical properties of the cell wall of corn root meristem and reported that there was a direct relationship between growth rate and mechanical properties of the cell wall. They also stressed that without directly measuring the mechanical properties of the cell wall, theories about the limiting role of a particular tissue in plant growth could not be confirmed. Of course, the physiological characteristics of seeds and agronomic traits are not independent of each other and there is a strong relationship between quality seeds and plant fertility (Pereira *et al*. 2016). Based on the results of our research, the existence of a significant relationship between GV, MGT, G%, SL and TSW from the group of seed and germination physiology traits with surface friction and adhesion traits in all three canonical variables showed that nanoscopic traits can have a high correlation with plant yield.

Table 6. Canonical correlations and their significant probability level for physiological variables group vs nanoscopic variables group.

	Correlation	Eigenvalue	Wilks statistic[a]	F	Num DF	Sig
1	0.972	17.237	0.000	3.582	66.000	0.000
2	0.924	5.824	0.009	2.434	50.000	0.000
3	0.839	2.374	0.061	1.782	36.000	0.025
4	0.756	1.331	0.207	1.415	24.000	0.153
5	0.664	0.789	0.482	1.071	14.000	0.415
6	0.372	0.160	0.862	0.481	6.000	0.814

[a] H0 for Wilks test is that the correlations in the current and following rows are zero.

Table 7. Set 1 Standardized canonical correlation coefficients for physiological variables.

Variable	1	2	3	4	5	6
TKW	-0.337	0.624	-0.006	-0.678	-0.058	0.658
GV	0.520	9.629	3.779	-1.514	-5.908	6.113
MGT	0.284	9.153	3.595	-1.537	-5.437	6.115
G%	-0.961	0.491	-0.017	-0.262	-0.008	-0.286
RL	0.159	-0.033	-0.243	-0.287	-0.431	-0.051
SL	-0.761	0.391	0.022	-0.595	-0.907	-0.238
WW	0.129	0.045	0.517	0.898	0.065	-0.402
DW	-0.134	0.242	0.119	-0.329	-0.071	0.477
TEW	-0.138	-0.388	0.233	-0.109	0.143	-0.598
TSW	-0.190	-1.061	0.705	-0.060	-0.156	-0.296
V	-0.229	-0.610	-0.153	0.545	-0.237	0.633

Table 8. Set 2 Standardized canonical correlation coefficients for nanoscopic variables.

Variable	1	2	3	4	5	6
LSF	0.724	1.416	-1.803	-0.763	1.303	-1.428
USF	-1.337	-0.688	0.121	1.815	-0.840	-0.187
LSA	-0.851	1.193	-0.466	0.267	1.154	-1.562
USA	1.090	-0.120	-0.383	0.949	-0.434	-0.975
LSE	-0.180	0.021	-0.298	-0.038	-1.521	-0.238
USE	-0.539	0.612	-0.053	0.248	0.849	1.234

CONCLUSIONS

Because the study of the relationships between nanomechanical and agronomic traits of the plant was conducted for the first time, the null hypothesis of the research was that there is no significant correlation between nanoscopic and agronomic traits. Based on the results, the null hypothesis was rejected and it was shown that the correlation rate is so high that it can be used for indirect selection in plant breeding programs and the results of this study can be used as a starting point for the development of plant breeding methods based on the nanomarker-assisted selection. The existence of structural relationships between nanomechanical traits and traits related to plant growth and development on the one hand, and the existence of a large number of plant tissues that can be studied by various methods of atomic force microscopy on the other hand, can be a good platform for more extensive research in the future.

ACKNOWLEDGEMENTS

The authors of the article would like to express their gratitude to Dr. Mohammad Ali Ebrahimi and Dr. Gholamreza Bakshi Khaniki for their kind advice in conducting this research.

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همبستگی‌های کانونی بین خواص نانو مکانیکی و برخی از صفات زراعی در چغندرقند

ا. تیموری یانه سری، ع. همتیان، د. داودی، و ف. روزبه

چکیده

هدف از این تحقیق بررسی رابطه بین خواص نانومکانیکی سطح و صفات زراعی در ارقام مختلف چغندرقند (*Beta vulgaris* spp.) بود. صفات زراعی مربوط به شاخص های مرحله جوانه زنی بذر و

مقاومت به ریزومانیا بود و در مقابل، گروهی از صفات نانومکانیکی تستای داخلی بذرها با استفاده از میکروسکوپ نیروی اتمی مورد بررسی قرار گرفتند. نتایج تجزیه و تحلیل همبستگی پارامتریک و ناپارامتریک بین صفات زراعی و نانومکانیکی نشان داد که وزن تر جوانه منفرد به ترتیب با اصطکاک سطح زیرین و چسبندگی تستای داخلی رابطه منفی و مثبت معنی‌داری دارد. به طور مشابه، وزن هزار پوسته بذر با کشش سطح بالایی همبستگی منفی و همچنین بنیه بذر با اصطکاک سطح بالایی همبستگی مثبت داشت. ضریب همبستگی اسپیرمن نشان داد که مقاومت به ریزومانیا نیز با چسبندگی سطح بالایی تستای داخلی همبستگی مثبت و معناداری دارد. سه متغیر کانونی بین دو گروه از صفات فیزیولوژیکی و نانومکانیکی معنی‌دار (R_1= ۰/۹۷۲ و FD= ۶۶، R_2= ۰/۹۲۴ و FD= ۵۰، R_3= ۰/۸۳۹ و FD= ۳۶) این صفات دارای نوعی رابطه علت و معلولی هستند و بنابراین قابلیت استفاده برای برنامه های اصلاحی و مطالعات سیستماتیک گیاهی را دارند.