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Automatic Phenotyping Test of Sunflower Seeds

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Abstract: The development of automated precision technologies for the phenotyping test of seeds by a complex of functional features in the selection process of sunflower is relevant and promising. The task of developing a device for the automatic phenotyping test of seeds and the algorithm for finding and isolating seeds based on color information was set. Research was conducted on a stand, which consisted of the following elements: Video Microscope Camera 1080 P 16MP HDMI USB manufactured by Eakins, a set of LEDs of three types (red, green, blue) and a personal computer.

The results of experimental studies of the process of automatic phenotyping test of seeds of different sunflower varieties allowed us to establish an average error of determining the geometric dimensions of sunflower seeds (length L and width B) – 0.06 mm. The histograms of the color distribution of sunflower seeds in the RGB color space with different illumination are established. As a result of the analysis of the obtained histograms of the color distribution of sunflower seeds in the RGB color space it is established that in the case of color homogeneity, the discreteness of the channels with red illumination is most clearly seen.

A device for automatic phenotyping test of seeds has been developed, which preserves the accuracy of individual measurement of the geometric dimensions of sunflower seeds, determining their shape and color, which corresponds to modern measuring tools, and provides low complexity and high technological implementation of the phenotyping test procedure (determination, ascertaining and identification) material, according to its morphological and marker features.

Keywords: seeds, sunflower, phenotyping, RGB, HSV, color index, device

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Introduction

Creation of new high-performance sunflower hybrids, environmentally stable and plastic under different conditions of cultivation with advanced immunity against diseases and pests, extremely long and costly process. Therefore, the systematization of breeding material by hereditary traits will significantly shorten the path and time of decision making and the mechanotronic systems of separation, selection and classification of material will significantly reduce the cost of the process (Shevchenko and Aliev, 2018).

The presence of stable external traits of the seeds (seed phenotype) of sunflower, which characterize the genetic features of this crop, allow the grouping of genetically valuable material for the purposes of breeding research (Kutishcheva *et al.*, 2015). Formed as a result of breeding work under the influence of soil and climatic conditions and features of cultivation of the trait of sunflower seeds can be carriers of especially valuable information (Kirichenko *et al.*, 2007).

In order to obtain a homogeneous genetic seed material of the parents, it is necessary to take into account the complexes of the signs and features of the seed. Sunflower seeds have a significant variety of geometric sizes, shapes, bulk weight, location of the center of gravity, pattern of surface and color. The length, width, thickness, shape and volume of the seed are quantitative traits that affect the productivity of the plant (Petrenkova *et al.*, 2004).

For example, the color of a sunflower achene varied from white to black through gray or brown and stripes forms. White color indicates the absence of phytomelanin, gray is enhances the black color, and in the presence of anthocyanin is formed coal-black color with black shimmer (Poliakova and Vedmedeva, 2016). White color dominates under all others. And black color dominates under the brown, brown color dominates under the gray (Gorohivets and Vedmedeva, 2016). For the breeding process, the color of achene, as a marker sign, plays a crucial role in identifying the appropriate sunflower variety, prevents falsification in its sale.

Taken into account the variety of seed colors of different varieties of the same crop, there is an urgent need for their identification and systematization (Atlas of morphological characteristics of sunflower varieties of annual *Helianthus annuus* L, 2011). Due to the fact that the perception of color for each person is individual, the exclusion of the human factor and the use of tools to recognize the color of the seeds are very relevant.

A high-throughput method to measure the area of seeds, using a desktop scanner and image analysis software to automate labor-intensive tasks (Herridge *et al.*, 2011). The disadvantages of this software: measures only the area of a seed, not the shape parameters, the lack of a function for determining the color of seeds.

Software SmartGrain uses a new image analysis method to reduce the time taken in the preparation of seeds and in image capture. Outlines of seeds are automatically recognized from digital images, and several shape parameters, such as seed length, width, area, and perimeter length, are calculated. (Tanabata *et al.*, 2012). The disadvantages of this software: the lack of a function for determining the color of seeds, with other lighting, poor ability to allocate seeds in the field, not a high speed of image processing.

The robot system «phenoSeeder» consists of a pick-and-place robot and a modular setup of sensors that can be versatilely extended. Basic biometric traits detected for individual seeds are two-dimensional data from projections, three-dimensional data from volumetric measures, and mass, from which seed density is also calculated. Each seed is tracked by an identifier and, after phenotyping, can be planted, sorted, or individually stored for further evaluation or processing (Jahnke *et al.*, 2016). The disadvantages of this system: the lack of a function for determining the color of seeds, throughput is limited, high labor costs.

So, it can be argued that the development of automated precision technologies for the phenotyping test of seeds by a complex of functional features in the selection process of sunflower is relevant and promising.

Materials and methods

Because of the above mentioned perspective, the task of developing a device for the automatic phenotyping test of seeds and the algorithm for finding and isolating seeds based on color information was set.

Research was conducted on a stand (Figure 1), which consisted of the following elements: Video Microscope Camera 1080 P 16MP HDMI USB manufactured by Eakins, a set of LEDs of three types (red, green, blue) and a personal computer. The principle of operation was as follows: by switching on the LEDs, the operator sets the illumination of a certain color, and the camera captures the stationary position or during their movement the seeds and transmits the received data to a personal computer.

The software algorithm consists of the following steps: obtaining an image from a camera; translating an image from the RGB (red, green, and blue colors) color space into HSV (hue, saturation, lightness); filtering by color (adjusting the

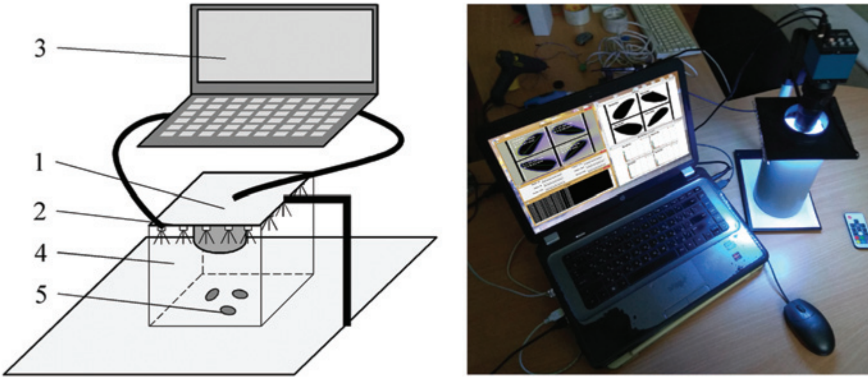

Figure 1: Schematic diagram (a) and General view (b) of an experimental device for automatic phenotyping test of seeds [1 – Video Microscope Camera 1080 P 16MP HDMI USB made by Eakins; 2 – sets of LEDs of three types (red, green, blue); 3 – personal computer; 4 – a protective screen; 5 – seeds].

color mask); performing basic morphological transformations – stretching and narrowing; finding the contours of a found object; tagging.

To implement the algorithm, it was decided to write a program using the OpenCV library. Different seeds of different colors, sizes and shapes were fed into the video stream's entrance. As noted, a special color mask must be customized for each seed. To highlight the desired color, you must select the boundaries of component H. The parameter S is responsible for color saturation. V Determines the brightness of the color. The shaded object will have a low value of V. When running the software of the device for automatic phenotyping test of seed material, 5 windows appear (Figure 2). The first window (Camera) – the original image from the camera in white illumination (all LEDs are on) with selected contours of seeds and their geometric dimensions. Second window (Filter) – Camera image after converting color image from HSV color space to black and white mask. In this mask, all pixels that fall into a given range turn white. Others are black. The third window (HSV) – HSV sliders, with which to adjust the color mask. The fourth window (RGBbarchart) – histograms of color distribution in the RGB color space. The Fifth Window (CMD) is a window for inputting and outputting messages and data.

The object of study was selected sunflower seeds of the following varieties of the collection of the Institute of Oil Crops of the NAAS: APS04, HA300B, HAP7, I2K87, InK1276, LD4, AH70029RF, APS10, HA298, I2K670, I3K1070, In18906, InK85, InK1124, InK2830, LD722, LD723, M19, RHA273, SL2966, KG13,

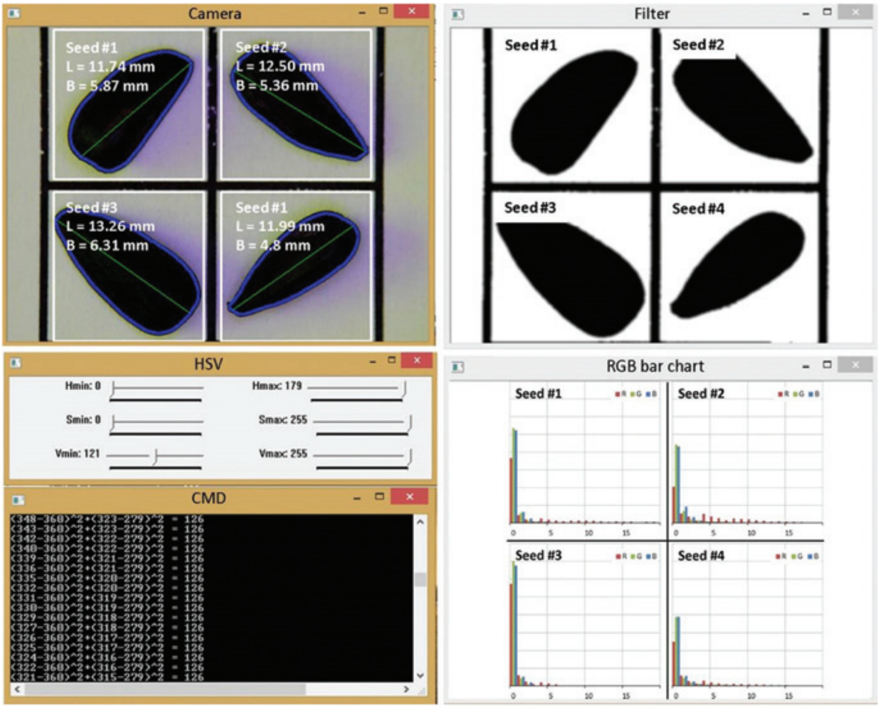

Figure 2: Device software for automatic phenotyping test of seed.

KG15, KG111, KG113, L7242, L259524, SL1218, SL2354, I2K20031, In7034, In18917. Selected seeds of sunflower varieties differed in marking traits, such as in color.

Lighting was chosen as a research factor, which was set by switching diodes on or off of a particular color. Four types of illumination were adopted for the studies: red (R = 255, G = 0, B = 0), green (R = 0, G = 255, B = 0), blue (R = 0, G = 0, B = 255) and white (R = 255, G = 255, B = 255).

To highlight a clear area (border) of seeds in the black and white image window of the developed software, ranges of HSV color space scales were set. The criteria for identifying seeds are their geometric dimensions (length L, width B and their ratio) and histograms of the color distribution of the seed regions in the RGB color space. As a result of the studies, it was necessary to establish an error in the measurement of geometric dimensions, which was determined by comparing the obtained digital data with the micrometer 1 accuracy class (±2.0 microns). In addition, a generalized universal indicator of seed color should be determined.

Results and discussion

As a result of studies, the values of the geometric dimensions of sunflower seeds (length L and width B) were determined by using an experimental device for automatic phenotyping test of seeds and micrometers. A graphical interpretation is presented at Figure 3. The average measurement error is 0.06 mm. According to the calculated Pearson criterion (χ^2), the normality of the error distribution of measurements is 2.21, more than the table value of χ^2 (0.95; 5) = 1.15. The hypothesis of the homogeneity of values by Kochren's criterion is tested $G = 0.14 < G_{0.05}$ (1; 100) = 0.33. The calculation of the coefficients of the calibration characteristics of the measurement of geometric dimensions using an experimental device for automatic phenotyping test assuming that this dependence is linear was tested using the Fisher criterion $F = 1.15 < F_{0.05}$ (91, 100) = 1.39.

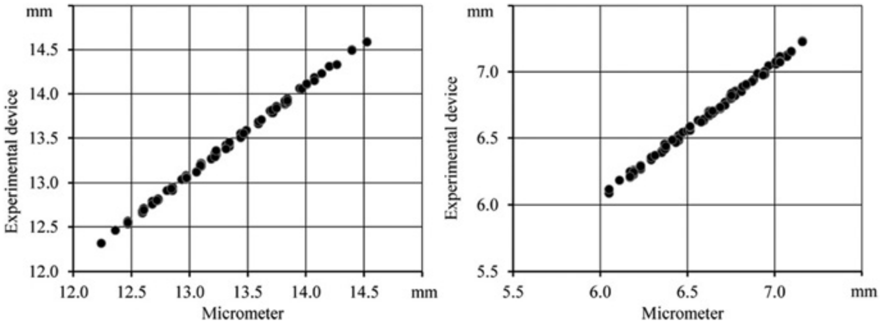

Figure 3: Comparison of geometric values of sunflower seeds; [(a) length L, and (b) width B] – determined using an experimental device for automatic phenotyping test of seeds and micrometers.

Histograms of the color distribution of sunflower seeds in the RGB color space with different illumination are presented at Figures 4–6.

As a result of the analysis of the obtained histograms of the color distribution of sunflower seeds in the RGB color space it is established that in the case of color homogeneity, the discreteness of the channels with red illumination is most clearly seen. The histogram has one maximum for each channel, which shifts depending on the color of the seeds. So for black color R = 182–189, G = 194–202, B = 211–218, and for white R = 112–118, G = 124–129, B = 133–139. Summarization of the results is given in the Table 1.

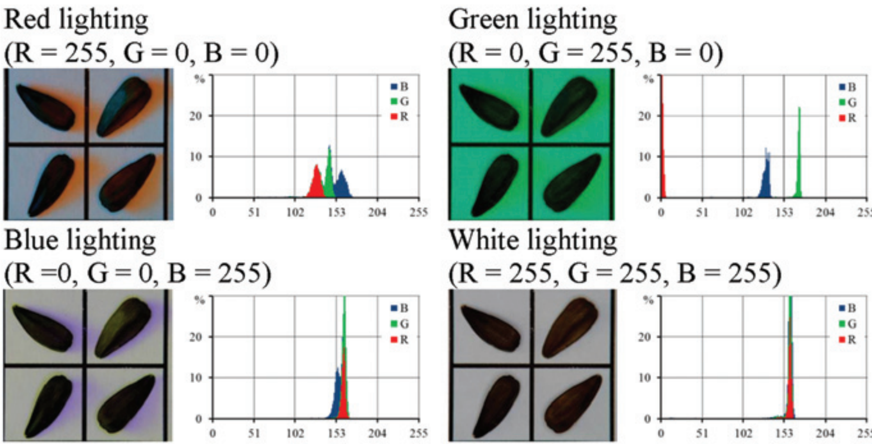

Figure 4: Histograms of color distribution of RHA273 varieties of sunflower seeds in RGB color space.

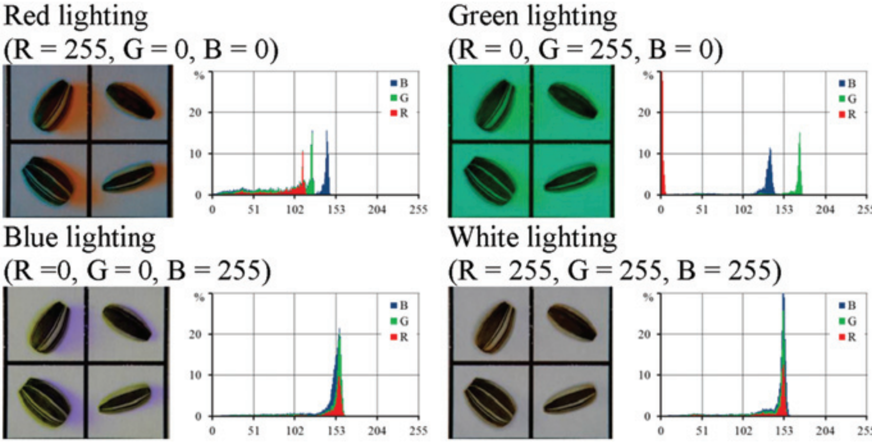

Figure 5: Histograms of color distribution of I2K20031 varieties of sunflower seeds in the RGB color space.

It was also found that in the case of two-color staining of sunflower seeds in the histograms of Figures 4–6 there are two maxima, each of which corresponds to one of the colors. The frequencies of these maxima correspond to the intensities of each color.

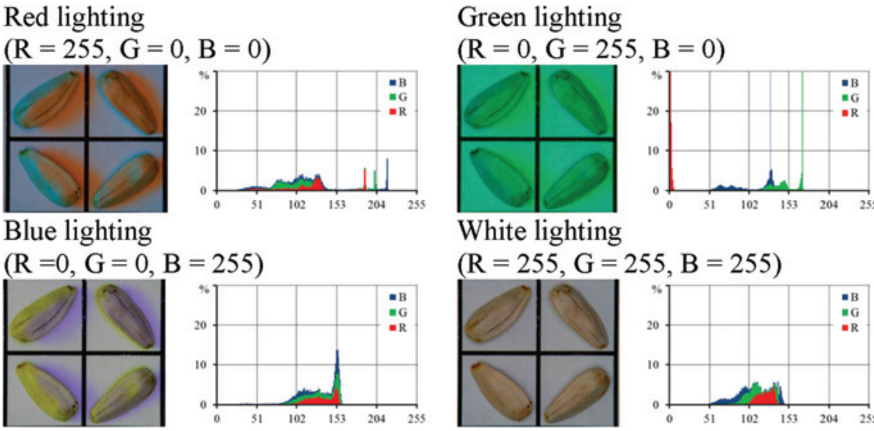

Figure 6: Histograms of color distribution of In18917 varieties of sunflower seeds in RGB color space.

So, we can determine the color index of seeds C, as a matrix of frequencies f corresponding maxima (max) in the color space RGB with red illuminated seeds:

$$C = \begin{pmatrix} R_{1\max} & f_{R1\max} & R_{2\max} & f_{R2\max} \\ G_{1\max} & f_{G1\max} & G_{2\max} & f_{G2\max} \\ B_{1\max} & f_{B1\max} & B_{2\max} & f_{B2\max} \end{pmatrix}, \tag{1}$$

where R, G, B – red, green, and blue colors, 0–255 units; f – frequency corresponding to the maxima, %; index «max» – maximum; index «1» and «2» – maximum number.

The histogram of the values of the channel maxima in the RGB color space with red illumination of sunflower varieties is presented at Figure 7.

A comparison of visual inspection with the results of determining the color of some sunflower seeds under red light is presented in Table 2. If there are monochromatic seeds, then the frequencies of the second color index maximum are 0. If they are striped, then the RGB color takes on the value of the second color. In practice, the color index of seeds C can be used as an accurate objective assessment of the color of the seed. With its help, it is possible to determine the marker characteristics of seeds in the process of reproduction.

To solve the problem of bioinformatic data analysis, evaluation of their quality, sorting of seeds by marker traits in oilseeds, selection by given traits at the initial stages of the breeding process, a device for automatic phenotyping test of sunflower seeds was developed (Figure 8).

Table 1: Generalization of the results of determining the color of sunflower seeds under different illumination.

Sample	Lighting	Red			Green			Blue			White		
	Color	R	G	B	R	G	B	R	G	B	R	G	B
APS04	units	126	151	170	2	182	147	168	170	160	160	160	161
	%	6.42	10.0	6.3	49.4	19.5	12.5	14.0	14.3	9.5	19.5	24.1	21.2
HA300B	units	132	161	182	2	181	145	169	170	173	169	170	173
	%	5.36	9.4	7.2	46.5	14.7	9.2	10.6	12.1	10.9	10.6	12.1	10.9
HAP7	units	127	144	166	2	185	154	170	173	164	167	167	167
	%	5.80	9.5	6.6	45.4	13.4	9.8	9.7	10.0	8.8	17.9	19.8	14.3
I2K87	units	117	131	147	2	167	128	156	158	151	154	154	158
	%	4.91	9.1	6.1	45.5	15.6	8.2	8.3	9.6	6.6	7.9	8.6	7.7
InK1276	units	127	150	166	2	175	136	171	174	155	164	164	164
	%	7.26	9.3	6.9	51.7	15.4	12.2	11.4	12.5	7.1	14.7	17.6	16.9
LD4	units	124	142	160	2	170	133	161	163	156	158	158	160
	%	5.94	8.2	5.5	46.7	15.1	9.0	10.0	11.6	10.1	8.9	11.5	12.4
AH70029RF	units	124	142	155	2	179	144	161	162	153	141	142	144
	%	5.89	8.7	6.4	56.7	20.5	9.6	12.4	14.5	9.3	11.3	14.2	13.1
APS10	units	118	136	151	2	178	144	164	165	154	155	155	156
	%	7.46	9.9	7.2	51.3	21.7	10.5	15.1	15.2	7.8	14.1	15.8	12.7
HA298	units	121	131	140	2	168	128	159	161	149	155	154	154
	%	5.85	9.8	6.3	56.9	23.6	9.8	11.3	11.1	7.7	11.8	12.6	13.0

(*continued*)

Table 1: (*continued*)

Sample	Lighting	Red			Green			Blue			White		
	Color	R	G	B	R	G	B	R	G	B	R	G	B
I2K670	units	125	145	169	2	175	137	166	167	164	164	164	166
	%	4.04	8.2	6.7	42.9	12.9	9.9	9.8	10.6	11.1	9.8	12.7	13.7
I3K1070	units	127	138	149	2	177	138	160	162	150	154	154	155
	%	7.10	12.2	6.0	54.0	24.8	11.5	13.6	15.8	8.7	17.7	18.2	17.1
In18906	units	130	145	162	2	175	137	162	164	156	164	164	165
	%	6.13	8.1	6.5	43.7	16.6	10.2	10.0	12.0	8.7	12.1	13.9	12.3
InK85	units	119	127	140	2	175	138	159	161	149	153	153	154
	%	7.27	10.6	7.2	52.7	21.4	11.8	11.6	14.3	8.1	16.0	17.7	14.0
InK1124	units	115	124	133	2	171	132	155	156	150	141	141	144
	%	8.36	13.3	7.6	51.4	29.8	14.5	11.2	10.9	10.8	12.0	13.1	12.3
InK2830	units	114	124	139	2	173	135	154	156	152	147	147	150
	%	6.51	13.5	7.6	58.1	22.9	18.6	10.3	11.2	13.0	12.9	14.1	13.3
LD722	units	122	140	160	2	169	134	163	164	161	157	157	161
	%	3.67	4.9	4.9	44.3	9.3	7.1	5.8	7.2	7.9	4.2	5.9	7.2
LD723	units	119	133	152	2	175	136	161	163	152	149	150	151
	%	3.52	7.4	6.1	53.1	11.8	8.7	7.2	10.2	6.1	6.4	11.0	11.4
M19	units	125	131	138	2	166	121	161	161	154	136	136	136
	%	8.17	9.8	6.5	56.9	22.5	8.4	11.3	13.0	10.5	13.8	16.9	14.7
RHA273	units	130	145	160	2	172	131	163	165	155	161	161	162
	%	8.16	12.2	6.9	58.0	22.3	12.1	17.5	16.9	11.6	24.8	26.3	19.3

SL2966	units	140	163	178	2	191	156	174	177	166	165	166	166
	%	4.34	8.1	6.4	46.9	7.6	7.1	5.5	6.9	6.5	8.3	10.1	9.0
KG13	units	135	152	169	2	179	140	170	172	163	165	165	166
	%	8.02	9.7	8.1	51.4	20.0	11.3	14.7	13.7	10.5	18.3	21.5	15.8
KG15	units	128	142	158	2	172	135	159	162	151	161	161	161
	%	6.26	9.8	6.2	58.8	17.7	15.9	8.6	10.0	6.8	13.5	15.1	13.9
KG111	units	130	147	160	2	175	137	166	166	159	164	164	164
	%	5.75	8.8	6.5	53.0	15.3	10.2	12.6	11.7	7.0	15.2	16.8	16.3
KG113	units	122	132	142	2	176	138	161	163	156	152	152	152
	%	7.04	10.1	6.9	57.2	25.2	9.9	15.5	17.0	10.8	15.7	17.7	15.2
L7242	units	132	157	176	2	189	157	174	177	164	161	161	161
	%	4.86	7.3	7.2	48.2	5.5	6.5	7.3	8.0	6.1	10.0	10.5	9.3
L259524	units	129	135	147	2	170	127	163	165	158	161	161	161
	%	5.51	6.6	5.5	57.9	15.2	7.9	10.2	10.5	9.2	14.7	16.4	16.2
SL1218	units	112	128	140	2	182	145	166	168	161	146	146	146
	%	6.22	9.0	5.3	48.1	17.5	10.8	10.4	12.5	11.0	13.6	15.3	13.1
SL2354	units	130	157	178	2	187	156	173	177	158	162	164	164
	%	3.75	5.7	6.3	44.7	5.9	6.2	5.2	5.9	4.4	4.8	6.0	5.7
I2K20031	units	113	124	142	2	173	136	157	159	153	153	152	155
	%	10.5	15.0	15.6	56.3	15.2	11.3	9.7	11.5	8.7	12.6	13.9	10.8
In7034	units	122	138	153	2	168	128	155	156	146	155	155	157
	%	11.5	14.6	18.0	48.9	21.5	11.1	10.1	11.1	8.6	12.0	13.5	12.7
In18917	units	189	202	218	2	171	130	153	155	153	134	139	142
	%	5.15	4.8	8.0	73.6	30.5	33.2	4.0	5.2	6.2	4.4	4.2	4.4

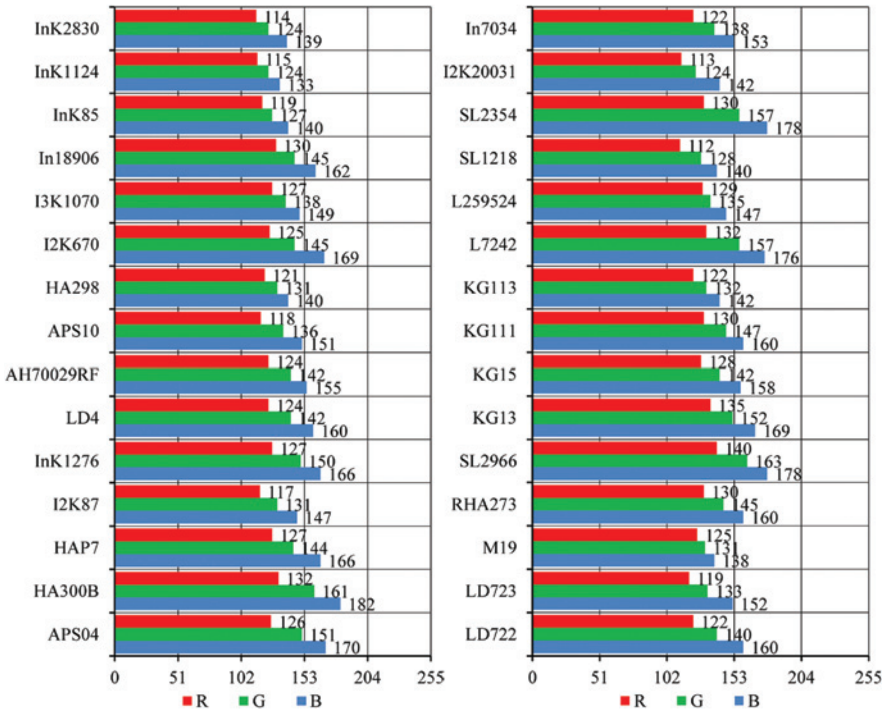

Figure 7: Histogram of maximums of channels in RGB color space with red illumination of sunflower varieties.

As a result of a production check, the automatic sunflower seed phenotyping test device had a capacity of 0.3 kg/h and a power consumption (including a personal computer) of 114 watts. During the inspection, a database of morphological and marker traits of 31 varieties of sunflower seed was created. Device for automatic phenotyping test of sunflower seeds provides low complexity and high technological implementation of the procedure of phenotyping (ascertaining and identification) of seeds, as breeding material, according to its morphological and marker traits.

Conclusions

The results of experimental studies of the process of automatic phenotyping test of seeds of different sunflower varieties allowed us to establish an average error

Table 2: Comparison with the results of visual inspection determine the color of sunflower seeds with red illumination.

Sample	Visual inspection		Color index of seeds C			
	Coloring	Continuity	R_{1max} G_{1max} B_{1max}	f_{R1max} f_{G1max} f_{B1max}	R_{2max} G_{2max} B_{2max}	f_{R3max} f_{G3max} f_{B3max}
Sl2613	black	monochromatic	182	9.65	0	0
			194	9.32	0	0
			211	9.25	0	0
KG13	dark brown	monochromatic	135	8.02	0	0
			152	9.72	0	0
			169	8.14	0	0
LD4/1	light brown	monochromatic	122	8.22	0	0
			142	9.14	0	0
			160	8.25	0	0
HA300B	black with gray stripes	striped	132	5.36	20	0.63
			161	9.44	13	0.19
			182	7.16	42	0.10
SL2966		striped	140	4.34	22	0.94
			163	8.07	42	0.34
			178	6.36	41	0.26
SL1218		striped	112	6.22	12	0.29
			128	9.05	22	0.37
			140	5.29	38	0.33
I3K1070		striped	127	7.10	40	0.17
			138	12.18	42	0.24
			149	6.01	33	0.14
I2K670	black with light gray stripes	striped	125	4.04	42	0.64
			145	8.19	30	0.62
			169	6.66	42	0.33
HA298	black with red stripes	striped	121	5.85	36	1.22
			131	9.79	13	0.41
			140	6.28	41	0.35
SL2354	burgundy	monochromatic	130	3.75	0	0
			157	5.66	0	0
			178	6.29	0	0
KG111	black with dark red stripes	striped	130	5.75	11	0.53
			147	8.84	34	0.12
			160	6.53	2	0.18

(*continued*)

Table 2: (*continued*)

Sample	Visual inspection		Color index of seeds C			
	Coloring	Continuity	R_{1max} G_{1max} B_{1max}	f_{R1max} f_{G1max} f_{B1max}	R_{2max} G_{2max} B_{2max}	f_{R3max} f_{G3max} f_{B3max}
InK1124	dark sire with brown stripes	striped	115	8.36	35	0.17
			124	13.29	25	0.11
			133	7.55	30	0.12
I2K2218	white	monochromatic	112	8.34	0	0
			124	8.31	0	0
			135	8.33	0	0

of determining the geometric dimensions of sunflower seeds (length L and width B) – 0.06 mm. The calculation of the coefficients of the calibration characteristics of the measurement of geometric dimensions using an experimental device for automatic phenotyping test on the assumption that this dependence is linear was verified using the Fisher criterion $F = 1.15 < F_{0.05}$ (91. 100) = 1.39.

The histograms of the color distribution of sunflower seeds in the RGB color space with different illumination are established. As a result of the analysis of the obtained histograms of the color distribution of sunflower seeds in the RGB color space it is established that in the case of color homogeneity, the discreteness of the channels with red illumination is most clearly seen. The histogram has one maximum for each channel, which shifts depending on the color of the seeds. So, for black color R = 182–189, G = 194–202, B = 211–218, and for white R = 112–118, G = 124–129, B = 133–139. It has also been found that in the case of two-color staining of sunflower seeds, the histograms show two maxima, each of which corresponds to one of the colors. The frequencies of these maxima correspond to the intensities of each color. That’s why it is possible to determine the color index of the seeds C as a matrix of frequencies f corresponding maxima (max) in the color space RGB with red illuminated seeds.

A device for automatic phenotyping test of seeds has been developed, which preserves the accuracy of individual measurement of the geometric dimensions of sunflower seeds, determining their shape and color, which corresponds to modern measuring tools, and provides low complexity and high technological implementation of the phenotyping test procedure (determination, ascertaining and identification) material, according to its morphological and marker features.

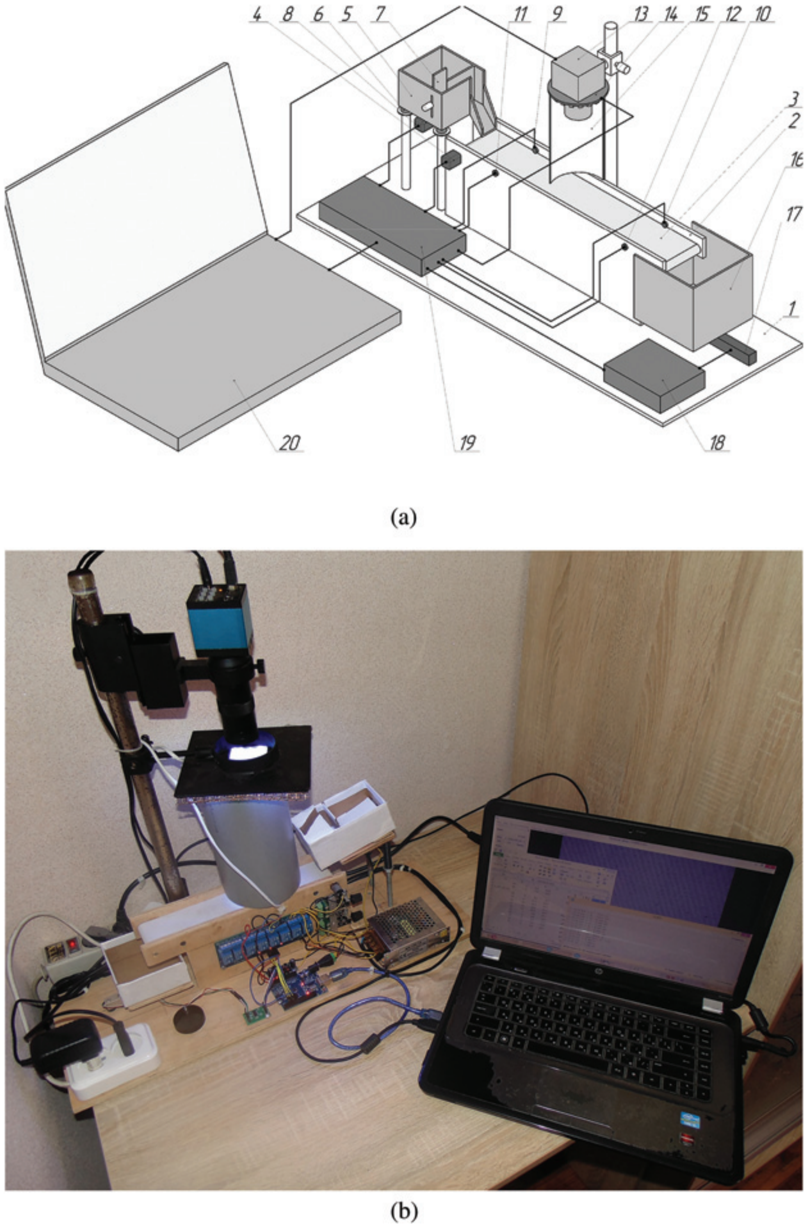

Figure 8: Scheme (a) and general view of the model sample (b) of a device for automatic phenotyping test of seeds [1 – frame, 2 – belt conveyor, 3 – tape, 4 – motor, 5 – seed tray, 6 – rubber shock absorbers, 7 – adjustable damper, 8 – vibration motor, 9, 10 – infrared LEDs, 11, 12 – photodetectors, 13 – camera, 14 – RGB LEDs, 15 – tube of light-proof material, 16 – receiving tray, 17 – strain gauge, 18 – amplifier, 19 – control unit, 20 – personal computer].

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