Moisture-dependent physical and physiological properties of accelerated aged pea (*Pisum sativum* L.) seeds

Manoj Kumar Mahawar[1,*], D. V. K. Samuel[2], J. P. Sinha[2] and Kirti Jalgaonkar[1]

[1]Horticultural Crop Processing Division,
ICAR-Central Institute of Post Harvest Engineering and Technology, Abohar 152 116, India
[2]Division of Agricultural Engineering,
ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

The present study was carried out to determine the physical as well as physiological properties of three fresh pea seed lots (cv. *Arkel*) with moisture content and germination percentage varying from 14.94% to 28.04% dry basis and 80% to 60% respectively. This variation in moisture content and physiological parameters was obtained using accelerated aging (40°C and 100% RH). The geometric (spatial dimensions, sphericity and surface area), gravimetric (terminal velocity, true density, test weight, bulk density and porosity), frictional (angle of repose, coefficient of static friction), mechanical (compressive strength) and physiological parameters (seedling dry weight, seedling length, vigour indices, electrical conductivity and root growth parameters) were determined for the selected seed lots. The effect of moisture content on seed lots was significant ($R^2 \geq 0.947$) on physical and physiological properties of seed lots. This study may help in designing seed priming prototype suitable for pea seeds.

Keywords: Moisture, physical properties, germination properties, accelerated aging.

PEA (*Pisum sativum* L.) is one of the important leguminous crops grown as a vegetable and generally consumed in fresh form. Simultaneously, food industries such as the canning industry also use pea seeds as a raw material for various applications. Increased consumption of good quality pea seed in the form of various food products, vegetables, feed and green manure, etc. has substantially raised its demand[1]. Among the group of pulse crops, field pea constitutes a major part of the production with a production of 10.85 million metric tonnes in an area of about 7.09 million hectares[2].

Determination of physical properties of any particular crop is essential for understanding and modulating its behaviour, which is required for the fabrication of relevant processing machineries. Evaluation of physiological properties becomes a prerequisite for understanding the effect of prolonged storage or accelerated ageing on seeds lots.

*For correspondence. (e-mail: manojmahawar362@gmail.com)

Several studies have determined the physical properties of crop seeds, for example, quinoa (*Chenopodium quinoa* wild) seeds[3], fenugreek (*Trigonella foenum-graceum*) seeds[4], jatropha (*Jatropha curcas* L.) seeds[5], melon (*Cucumis melo* L.) kernels and seeds[6] as a function of their moisture content. However, detailed information on the correlations between the moisture and physical as well as physiological properties of pea seeds subjected to accelerated ageing has not been reported. A better understanding of these parameters is required for the effective design and development of seed quality enhancement prototypes. Therefore, the objective was framed accordingly to predict the impact of moisture variation on physical and physiological properties of aged pea seeds. Different parameters, viz. spatial dimensions, sphericity, surface area, true density, bulk density, porosity, terminal velocity, thousand seed mass, angle of repose, coefficient of static friction, compressive strength, seedling length, seedling dry weight, vigour index-I, vigour index-II, electrical conductivity and root growth parameters were determined. The regression models were developed for each dependent parameter with respect to moisture content. This comprehensive study should be considered a preliminary study which further developed a seed priming prototype/equipment suitable for pea seeds.

Hot air oven method (105 ± 1°C for 24 h) was used to determine the moisture content of seeds[7]. Standard germination test for pea seeds was used to determine germination percentage by wrapping the known number (400 nos) of seeds in a wet germination towel and keeping them in a germination chamber (20 ± 1°C and 80 ± 5% RH). Final germination count of all the seed samples was taken after the eighth day[8].

Pea seed must have 75% minimum germination to maintain physical and genetic purity[9]. Keeping this in mind, three seed lots were prepared with the aim that among the lots, one lot was of minimum germination percentage, and one lot each below and above minimum germination percentage. Such a lot selection will help in effective understanding of the beneficial effects of hydropriming on seed quality attributes of pea seeds. This was reported in another study conducted by the author[10]. To attain the required seed lots for the experiment, initially two lots of pea seed (cv. *Arkel*) were procured. One lot was sourced from the National Seeds Corporation, New Delhi, India and the other was from the ICAR-Indian Agricultural Research Institute (IARI) regional station, Karnal, Haryana, India. The germination percentage for these seed lots was found to be approximately 80% and 75% respectively, while the moisture content was 14.94% (d.b.) and 14.4% (d.b.) respectively. The procured seeds were subsequently brought to the laboratory for preliminary cleaning to remove unwanted substances like dirt, dust, chaff, stones, broken and immature seeds. Further, to achieve the desired difference in germination percentage, the available (80% and 75% germination) seed lots were subjected to accelerated ageing by covering 250 g of seeds with a muslin cloth and placing them in a desiccator (40 ± 1°C, 100% RH)[11]. After 24 and 48 h, certain number of seeds were withdrawn from both the lots and the moisture content and germination percentage were determined. This practice enabled two more lots to evolve from the existing seed lots. Out of these, three seed lots with different moisture levels were selected, thus simultaneously achieving the criterion of variation in germination percentage as per the minimum germination standards. The nomenclature of selected seed lots was: L_1 (80% germination with 14.94% d.b. moisture), L_2 (70% germination with 25.47% d.b.) and L_3 (60% germination with 28.04% d.b.). The same information has been tabulated in Table 1.

The average size of twenty randomly selected seeds from each lot was computed by measuring primary linear dimensions, specified as thickness (T), width (W) and length (L) using digital vernier callipers (Mitutoyo (Japan), least count 0.01 mm. Geometric mean diameter, GMD (D_p) and sphericity (Φ_S) were also computed using the relationship given by Mohsenin[12]

$$D_p = (L \times W \times T)^{1/3}, \tag{1}$$

$$\Phi_S = \frac{(LWT)^{1/3}}{L}, \tag{2}$$

the surface area (S) of the samples was calculated by keeping the equivalence with a sphere having identical GMD, using eq. (3) as described by McCabe *et al.*[13]

$$S = \pi D_p^2. \tag{3}$$

where S is surface area (mm^2); D_p the geometric mean diameter (mm). The interpretation of density values of seeds helps in designing of storage bins, silos, removal of impurities from the desired material, cleaning, grading and product quality evaluation[14]. Density was measured using a wooden box with inside dimensions of 100 × 100 × 100 mm. Seeds were poured into the box and excess seeds were removed by rolling a measuring scale on the rim of the box without compaction[15]. The weight of the box containing the seeds was noted with five replications and the average bulk density calculated by dividing the weight of the seeds by the volume of the box. True density of the seeds is considered useful for designing proper separation equipment and was estimated using toluene displacement method. The known quantity of seed was dipped in toluene and the volume of toluene displaced was recorded[12]. Porosity of the grain signifies the resistance to air flow during aeration and drying operations[16]. Porosity was calculated using the following relationship[12]

$$\varepsilon = 100(1 - \rho_b/\rho_t), \tag{4}$$

where ε is porosity (%), ρ_b the bulk density (g cm^{-3}) and ρ_t is true density (g cm^{-3}). Test weight (1000 seeds) was determined using a digital balance (Goldtech, India, least count 0.001 g). Initially 100 seeds from the bulk sample were randomly chosen and weighed. Average weight of five replications was calculated. Further, the reading was extrapolated to the weight of 1000 seeds.

The terminal velocity was measured using an experimental set-up consisting of a mild steel box fitted with a long glass tube, blower and a transformer to regulate the voltage. Air velocity was measured using hot wire anemometer (Lutron, model AM-4204). To regulate the air velocity in a vertical glass tube, a throttle was provided with a blower. Seeds were poured from the top of the glass tube and the upward air velocity required to suspend the seeds was recorded for five samples. The mean value was then determined[17].

The ability of the seeds to flow is represented by predicting the angle of repose. The knowledge of angle of repose is important for designing hopper openings as well as storage structures during bulk handling of seeds[14]. For measurement, the seeds were dropped from a height of 200 mm on a circular plate (200 mm diameter) to form a natural heap. Height and diameter of the seed heap were noted to obtain the angle of repose using eq. (5) given below[18]

$$\Phi = \tan^{-1}(2H/D), \tag{5}$$

where H and D refer to height and diameter (in mm) of the heap respectively. Coefficient of static friction was measured by inclined plane method on plywood, mild steel and cast iron surfaces[19]. The seed sample was kept on an adjustable tilting plate which was raised steadily till the sample started to slide along the surface. The angle at which downward movement started was noted as α and the following relationship was used to determine μ

$$\mu = \tan \alpha, \tag{6}$$

frictional properties, viz. angle of repose and coefficient of static friction are considered to be important for designing seed containers and storage structures[3].

Table 1. Germination and moisture content of the seed lot after accelerated ageing

	Available seed lots (with 80% and 75% germination)			
	80%		75%	
Ageing duration	Day 1	Day 2	Day 1	Day 2
Germination (%)	(76%)	(70%)	(66%)	(60%)
Moisture (d.b., %)	19.76	25.47	20.77	28.04

Compressive strength is a vital mechanical property in relation to seed breakage. Evaluation of seed hardness with respect to relative changes in the seed coat after being subjected to accelerated aging becomes more relevant. Texture analyser (TA + HDi®, Stable Micro Systems, UK) was used to determine seed hardness using a stainless steel cylindrical probe of 75 mm diameter in compression mode. The compressive force was measured using the load cell of 50 kg capacity. A single seed from the test lot was positioned in the centre of the plate to carry out the observation. The peak force and displacement were estimated based on the time taken to reach the peak force. The first peak force in the force–distance diagram was taken as the hardness of the grain. The average value of ten observations was calculated for each seed lot.

After the final germination count, seedling length (SL) of ten random seedlings were computed using a scale and recorded in centimetres. Seedling dry weight (SDW) of the same ten seedlings was determined after allowing them to dry in a hot air oven (70 ± 5°C) till entire moisture was removed. The weight was expressed in gram per ten seedlings. Vigour indices (VI-I and VI-II) were determined[20]. The electrical conductivity (EC) of seed leachate was measured using the method suggested by Priestley[21].

To determine root growth parameters, the germinated seedlings were washed thoroughly with tap water without damaging the roots. Scanning and image analysis for root characteristics were carried out using Root Scanner (LA 1600). Root morphology as well as architecture measurements (total length, surface area, thickness and volume of root) were done using WIN RHIZO® program (Regent Instruments Inc., Canada).

Data obtained for various dependent properties (physical and physiological) were subjected to linear and nonlinear regression analysis using Microsoft Excel 2010 (ref. 6). The relationships showing the dependencies of the determined properties on moisture content were fitted to the respective models, viz. linear, logarithmic, exponential and power models and the empirical constants were determined in each case. Subsequent to the comparison, models with high coefficient of determination (R^2) were chosen.

Average values of the three axial dimensions (L, W, T) of the seeds behaved positively with increase in moisture content and were substantiated with prominent R^2 values (Figure 1). The correlations between moisture content and the spatial dimensions were logarithmic and exponential in nature, represented by the following equations

$$L = 0.732 \ln(M_C) + 7.896 \quad (R^2 = 0.987), \tag{7}$$

$$W = 0.636 \ln(M_C) + 6.427 \quad (R^2 = 0.969), \tag{8}$$

$$T = 5.732\, M_C^{0.1162} \quad (R^2 = 0.958), \tag{9}$$

$$D_p = 0.694 \ln(M_C) + 6.624 \quad (R^2 = 0.973). \tag{10}$$

The absorption of moisture by seeds while being subjected to accelerated ageing is the possible reason for this positive relation. Similar increase in axial dimensions of seeds as a result of moisture uptake was reported for different granular agro-materials such as rapeseed[22], dried pomegranate seeds[23], paddy rice[24], guar seeds[25] and kenaf seeds[26].

Sphericity of seed lots also exhibited an increasing trend with increase in moisture content and the values ranged from 0.838 to 0.854 (Figure 1). This might be due to proportional changes in the dimensions of seed lots. These findings were in consonance with the reports for fenugreek seeds[4] and pistachio kernels[27]. The relationship between sphericity and moisture content appears to be logarithmic as suggested by the regression equation

$$\Phi_s = 0.0147 \ln(M_C) + 0.838 \quad (R^2 = 0.996). \tag{11}$$

The surface area of seed lots followed an increasing pattern with increase in moisture content and the values ranged between 136.72 and 169.25 mm^2. The dependence of surface area on moisture was nonlinear and the same can be expressed mathematically as

$$S = 30.34 \ln(M_C) + 137.840 \quad (R^2 = 0.976). \tag{12}$$

The direct relation of surface area with the three spatial dimensions of seed lots is the pertinent reason for this increase. Similar results were reported for jatropha seeds[5], paddy rice[24] and red kidney bean grains[28].

The test weight of seed lots proportionally increased with increase in moisture content and varied from 210.28 to 217.03 g. This increase in test weight was attributed to the corresponding increase in axial dimensions of seed lots. The logarithmic equation for test weight can be formulated as

$$W_{1000} = 6.27 \ln(M_C) + 210.48 \quad (R^2 = 0.983). \tag{13}$$

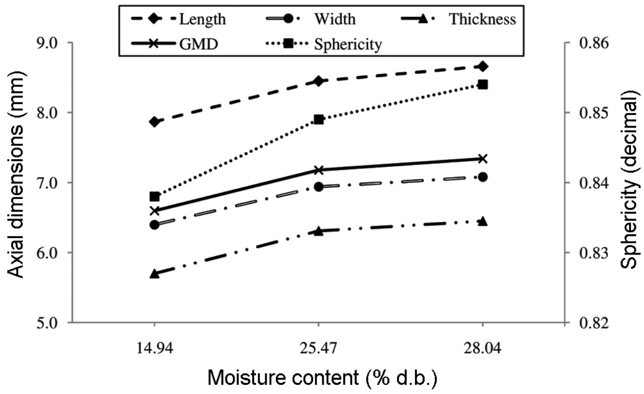

Figure 1. Variation of axial seed dimensions of aged pea seeds with moisture content.

Similarly, increasing trend in test weight with increase in moisture content was also reported for guar seeds[25] and *Moringa oleifera* seeds[29]. Graphical representation of the variation in surface area and test weight as a function of moisture content is shown in Figure 2. The variation in geometric properties of selected seed lots helped in designing the priming chamber with respect to its capacity and dimensions and selection of sieve mesh size[30].

The decrease in average bulk density from 0.702 to 0.608 g/cc with increase in moisture content from 14.94% to 28.04% d.b. was observed. This revealed that an increase in mass due to moisture absorption was comparatively lower for the seed samples than the associated volumetric expansion of the seeds[31]. Negative linear relationship between bulk density and moisture content was also reported in sorghum seeds[32] and pea seeds[33]. The mathematical relationship of bulk density with moisture content was linear and the same has been depicted below

$$\rho_b = -0.047M_C + 0.746 \quad (R^2 = 0.990). \tag{14}$$

The true density of seed lots reduced from 1.187 to 1.142 g/cc with increase in moisture content from 14.94% to 28.04% d.b. The rate of increase in volume of an individual seed was higher than the increase in its weight. This could be responsible for decrease in true density. The relationship between true density and moisture content of seed lots can be correlated as

$$\rho_t = -0.022M_C + 1.21 \quad (R^2 = 0.992). \tag{15}$$

Similarly, negative linear behaviour of true density was reported in karingda seeds[7], okra seeds[19] and pea seeds[33].

The porosity of seed lots varied from 40.85% to 46.76% with increase in moisture content. Swelling and expansion of seeds facilitated the increase in void space in the seeds as well as increase in volume, thereby increasing the porosity. The relationship between moisture content and porosity of pea seeds was appropriately fitted to a power model as follows

$$\varepsilon = 40.875\, M_C^{0.1234} \quad (R^2 = 0.999). \tag{16}$$

Similar findings were reported for jatropha seeds[5] and red kidney bean grains[28]. The pictorial trend of density values and porosity as a function of moisture content of seed lots is shown in Figure 3.

The terminal velocity recorded for seed samples ranged from 9.9 to 11.2 ms^{-1} and varied linearly with moisture content. This might be due to the weight gain in seeds with prolonged duration of ageing, resulting in further increase in air velocity needed to suspend the seeds. The mathematical relationship between terminal velocity and moisture content can be represented by the equation

$$V_t = 0.65M_C + 9.23 \quad (R^2 = 0.998). \tag{17}$$

A similar trend was recorded for karingda seeds[7] and kenaf seeds[26]. With respect to the design aspect of the seed priming prototype, the findings related to gravimetric properties were supportive in deciding the rotation speed of the seeds during hydropriming[30].

The peak compressive force at which seeds crack or break reduced from 42.38 to 15.22 N at the corresponding displacement of 0.5 mm with raised moisture content. Moisture uptake during accelerated ageing affected the hardness of the seeds. They became relatively softer in texture for higher moisture values. The linear trend between moisture content and compressive force can be represented as

$$F_c = -13.58M_C + 56.17 \quad (R^2 = 0.999). \tag{18}$$

This analogous type of deviation was reported for chick pea seeds[34] and dried pomegranate seeds[23]. Figure 4 shows the graphical representation of the observations related to terminal velocity and compressive strength.

Angle of repose improved from 32.66° to 36.33° with increase in moisture from 14.94% to 28.04% d.b. (Figure 5). This trend was due to the presence of moisture on the

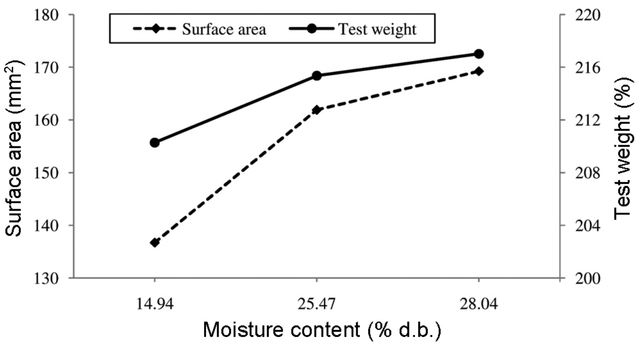

Figure 2. Effect of moisture content of aged pea seeds on surface area and test weight.

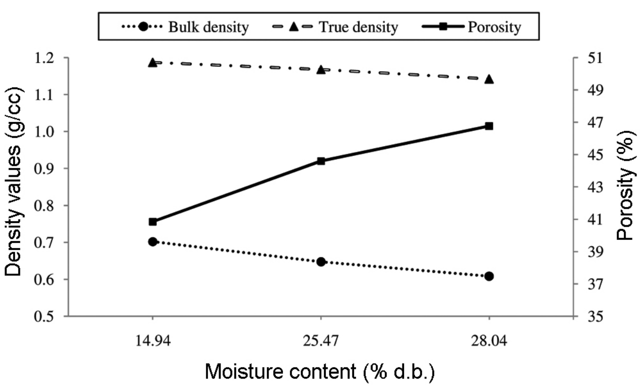

Figure 3. Variation of bulk density, true density and porosity with moisture content.

surface layer of seeds. The surface tension that occurs helps in binding the seeds together[31]. The analogous data trend for the angle of repose with moisture was validated in studies conducted for sorghum[32], jatropha[5] and karanja kernels[31]. The dependence was linear and can be represented by the equation

$$\Phi = 1.835M_C + 30.77 \quad (R^2 = 0.997). \tag{19}$$

As the moisture content increased, the seeds became rough and their sliding characteristics were diminished. The rough surface of plywood offered higher resistance to grain flow when compared to mild steel and cast iron, which were smoother. Thus, coefficient of friction was higher for plywood. The coefficient of static friction improved with moisture percentage for the selected three surfaces. The value ranged from 0.591 to 0.625 for plywood, 0.558 to 0.605 for mild steel and 0.541 to 0.589 for cast iron. Regression analysis revealed that a linear correlation exists between the coefficient of static friction and moisture content. This can be expressed mathematically using the equations

$$\mu_{pl} = 0.017M_C + 0.574 \quad (R^2 = 0.995), \tag{20}$$

$$\mu_{ms} = 0.023M_C + 0.534 \quad (R^2 = 0.999), \tag{21}$$

$$\mu_{ci} = 0.024M_C + 0.518 \quad (R^2 = 0.994). \tag{22}$$

The high frictional force between seeds and contact surface at high moisture content[35], attributed to the change in coefficient of static friction values. Analogous results were reported for rapeseed[22]. Graphical representation of the coefficient of static friction on the selected three surfaces against moisture content is presented in Figure 5. The information collected on the frictional properties of seed lots was helpful during the discharge of seeds from the priming chamber after hydropriming. All the above moisture related parameters of pea seed lots have been reported in Table 2.

Germination associated attributes of seed lots, viz. SL, SDW and vigour indices reduced with increase in exposure time to accelerated ageing. This was because physiological changes occurred within the seeds throughout the ageing process. However, EC values increased with reduction in germination. This was due to eruption of more leachate from the seed coat which was loosened during the process of ageing. Table 2 shows the variation in the values of SL, SDW, VI-I, VI-II and EC with respect to the moisture content of seed lots. The relationship between the dependent parameters and germination of seed lots can be represented by the equations

$$SL = 17.812\, M_C^{-0.199} \quad (R^2 = 0.997), \tag{23}$$

$$SDW = 0.298\, M_C^{-0.328} \quad (R^2 = 0.947), \tag{24}$$

$$VI\text{-}I = -513.6 \ln(M_C) + 1430.2 \quad (R^2 = 0.999), \tag{25}$$

$$VI\text{-}II = 24.12\, M_C^{-0.583} \quad (R^2 = 0.997). \tag{26}$$

$$EC = 1.568e^{0.4821}M_C \quad (R^2 = 0.997). \tag{27}$$

The germination attributes helped in understanding the change in quality attributes before and after hydropriming, while performing experiments with the prototype under controlled conditions of the process affecting parameters.

The root portion was scanned to evaluate the growth parameters with respect to ageing effect. The evaluated attributes collectively reduced with corresponding reduction in germination of seed lots which was due to ageing. The values of root surface area, volume, length and projected area were observed to be more for healthy seeds when compared to aged seeds (Table 2).

This study revealed the consistent relationships that showed the effects of moisture content on physical as well as physiological properties of aged pea seed lots. The study contributed significant knowledge on the

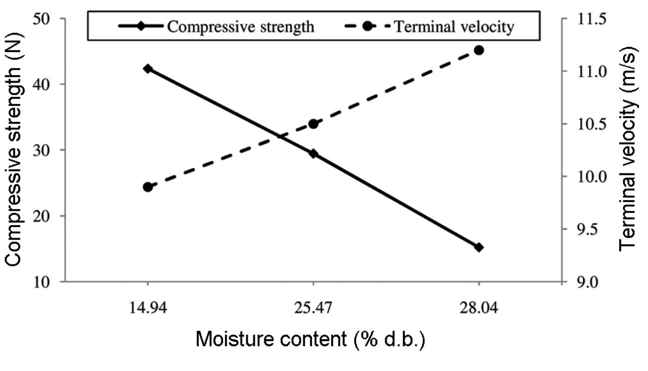

Figure 4. Variation of compressive strength and terminal velocity with moisture content.

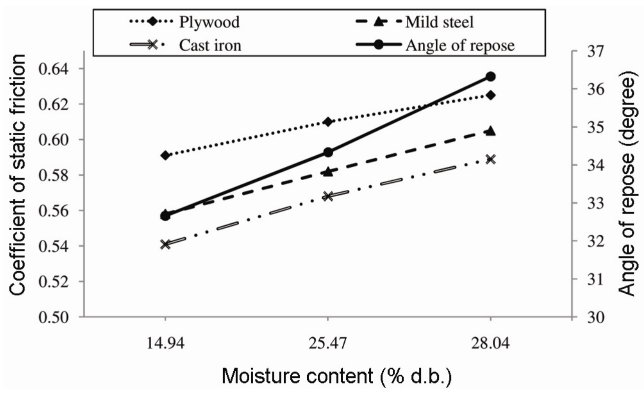

Figure 5. Effect of moisture content on coefficient of static friction and angle of repose.

Table 2. Variation of determined properties of the three selected seed lots

Lot	L_1	L_2	L_3
Physical properties			
Length (mm)	7.87 ± 0.016	8.45 ± 0.036	8.66 ± 0.037
Width (mm)	6.40 ± 0.163	6.94 ± 0.037	7.08 ± 0.029
Thickness (mm)	5.70 ± 0.216	6.31 ± 0.033	6.45 ± 0.029
D_p (mm)	6.597 ± 0.142	7.179 ± 0.011	7.340 ± 0.005
D_a (mm)	6.566 ± 0.130	7.233 ± 0.012	7.396 ± 0.007
Sphericity	0.838 ± 0.016	0.849 ± 0.002	0.854 ± 0.003
Surface area	136.75 ± 5.878	161.92 ± 4.52	169.258 ± 3.60
Test weight	210.28 ± 0.037	215.35 ± 0.029	217.03 ± 0.059
Bulk density	0.702 ± 0.04	0.647 ± 0.03	0.608 ± 0.04
True density	1.187 ± 0.05	1.168 ± 0.03	1.142 ± 0.06
Porosity	40.85 ± 0.045	44.6 ± 0.033	46.76 ± 0.022
Terminal velocity	9.9 ± 0.216	10.5 ± 0.163	11.2 ± 0.374
Compressive strength	42.38 ± 0.079	29.44 ± 0.139	15.22 ± 0.051
Angle of repose	32.6 ± 0.054	34.3 ± 0.077	36.3 ± 0.045
Germination properties			
Seedling length (cm)	17.85 ± 2.08	5.43 ± 1.42	14.37 ± 1.18
Seedling dry weight (g)	0.304 ± 0.03	0.226 ± 0.04	0.214 ± 0.05
VI-I	1428 ± 15.4	1080.1 ± 16.25	862.2 ± 11.19
VI-II	24.28 ± 3.66	15.82 ± 2.08	12.86 ± 1.56
EC (mS/cm)	0.154 ± 0.078	0.183 ± 0.045	0.207 ± 0.029
Root growth parameters			
Length (cm)	28.96 ± 4.72	25.17 ± 3.54	21.52 ± 2.98
Surface area (cm^2)	6.52 ± 1.54	5.86 ± 1.07	4.90 ± 1.12
Projected area (cm^2)	2.18 ± 0.36	2.09 ± 0.54	1.56 ± 0.78
Volume (cm^3)	0.14 ± 0.01	0.12 ± 0.007	0.09 ± 0.003

Values are mean ± S.D. of three replications.

changing behaviour of determined properties with moisture percentage. The values of different dependent parameters helped in deciding the design criteria of the seed priming prototype, such that the controlled conditions could be precisely maintained for all the three selected seed lots during the experiment. The observed relationship between the physical and germination properties with changing moisture content was best fitted with linear, logarithmic and power models for all dependent parameters except for EC, for which the exponential model was found appropriate. The gathered information may find applicability in the design and fabrication of seed processing equipment pertaining to cleaning, grading, separation, storage and quality enhancement (treatment, priming, etc.).

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Proline-rich proteins may regulate free cellular proline levels during drought stress in tomato

Ranjit Singh Gujjar, Suhas G. Karkute*, Ashutosh Rai, Major Singh and Bijendra Singh

Division of Crop Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi 221 305, India

Proline (Pro)-rich proteins (PRPs), initially identified as structural proteins of cell wall, have emerged as multifunctional plant proteins in recent past. Their vibrant role in plant development and environmental stress promoted us to study a *SlPRP* gene of tomato, which was significantly downregulated under drought stress in a microarray experiment performed in our laboratory. Promoter analysis of *SlPRP* revealed a number of stress-responsive protein-binding sites, confirming its expression in response to stress. Expression of *SlPRP* gene in different tissues of tomato, viz. root, stem, leaf and flower was studied to analyse the gene expression pattern in response to drought stress. Further, we have correlated the expression of *SlPRP* gene with Pro levels of the respective plant tissues under drought stress. In anticipation, it has been observed that downregulation of *SlPRP* gene is coupled with simultaneous increase in cellular Pro concentration in all the tissues under drought stress, except the roots. This could help preserve the available cellular proline to function as osmoprotectant during stress. The present results propose a hypothesis where PRPs may regulate free cellular proline levels during drought stress by regulating their own gene expression. Thus, it may be concluded that transcription of PRPs in plants is synchronized with the cellular Pro concentration under environmental stress in order to provide drought tolerance to plants.

Keywords: Drought stress, gene expression, proline-rich proteins, tomato.

PROLINE (Pro)-rich proteins (PRPs) are structural cell-wall proteins that were initially identified as wound-induced gene products in carrot storage roots[1]. Environmental stress or physical damage to plants also causes PRPs to accumulate in cell walls, whereas their expression is temporally regulated during plant development[2]. PRPs have been categorized into three classes. One of these has PRPs with several copies of the POVEKPOV**X**K motif[3], whereas the other two classes (HyPRPs and NHyPRPs) have PRPs with a hybrid structure. HyPRPs contain a repetitive proline-rich region at the N-terminal domain and a conserved eight-cysteine motif (8 CM) at the C-terminal domain[4,5]. In contrast, NHyPRPs have a C-terminal region with a high percentage

*For correspondence. (e-mail: S.Karkute@icar.gov.in)