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ORIGINAL ARTICLE

Optimization of pea (*Pisum sativum*) seeds hydropriming by application of response surface methodology

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Abstract Evaluation of selected parameters viz. initial germination percentage (IGP), soaking duration (SD), process temperature, rotation speed (rpm) and air flow rate (AFR) was performed in this research investigation for hydropriming of Pea (*Pisum sativum*) seeds. Three seed lots having difference in their moisture content (14.94–28.04 % d.b) and germination percentage (60–80 %) were selected in this study. Procured seed lots were subjected to variable duration of accelerated aging (40 ± 1 °C, 100 % RH) to attain necessary seed lots for experimental run. Response surface methodology (Box–Behnken design) with five factors and three-level combination was adopted, and the independent variables are germination percentage (80, 70, 60), soaking duration (45, 60, 75 min), temperature (20, 25, 30 °C), rotation speed (320, 340, 360 rpm) and air flow rate (0.411, 0.548, 0.685 m^3/min). Second order polynomial equation was fitted for analyzing the experimental data and data was also subjected to analysis of variance as a part of regression analysis. Process responses which were selected to evaluate the effect of hydropriming were moisture content after hydropriming, final germination percentage, seedling length, seedling dry weight, vigor indices (VI–I and VI–II) and electrical conductivity. Regression analysis suggested that models were significant for all process responses and using numerical optimization technique, the optimal solution found was 75 % IGP, 55 min SD, 20 °C temperature, 320 rpm and 0.50 m^3/min AFR. Values predicted by model were found to be at par with the results of a confirmation experiment carried out at optimum conditions.

Keywords Accelerated aging · Hydropriming · Response surface methodology · Optimization

Abbreviations

IGP	Initial germination percentage
T	Temperature
AFR	Air flow rate
ANOVA	Analysis of variance
FGP	Final germination percentage
SDW	Seedling dry weight
VI-II	Vigor index-II
d.b.	Dry basis
L_2	Seed lot 2
MT	Metric ton
RSM	Response surface methodology
mS/cm	Millisiemens/centimetre
SD	Soaking duration
rpm	Rotation speed
RH	Relative humidity
MC	Moisture content
SL	Seedling length
VI–I	Vigor index-I
EC	Electrical conductivity
L_1	Seed lot 1
L_3	Seed lot 3
Mha	Million hectare
BBD	Box–Behnken design
min	Minute

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Introduction

Pea (*Pisum sativum* L.) belongs to family *Fabaceae* is one among the leading leguminous crops in several other countries including India. It is predominantly consumed as fresh and also serves as raw material for food industries primarily involved in individual quick freezing of pea seeds. Utilization of superior quality pea seeds in various food products, vegetables, feed and green manure, etc., resulted in significant increase in its demand. On the contrary, the quality of seeds available in market of developing countries remains poor. Use of such seeds for sowing purpose not only becomes the reason for immense loss to farmers as well as seed corporations, but in addition it also has negative impact on the yield and quality parameters of crop (Guan et al. 2011). In India, pea is cultivated in 0.433 Mha area with production of 3.86 million MT and overall productivity of 8.9 MT/ha. Major pea producing states are Uttar Pradesh (39.5 %), Madhya Pradesh (12.9 %), Assam (7.2 %), Jharkhand (5.6 %), Himachal Pradesh (5.5 %) and West Bengal (5.0 %) (National Horticulture Board 2014).

Recent agricultural practices demands more favoritism towards being accurate and technology adaptive, and the insistence that every single seed must readily germinate and produce a vigorous seedling ensuring higher yield and greater monetary returns. Hence, the demand for only genetically, morphologically, pathologically and physiologically sound seed is competent enough of increasing the productivity (Abdolahi et al. 2012). Seeds must also possess enhanced storability to make sure of the generation of good quality crop during the coming season. But during the storage period, seeds start losing its vigor and viability and sowing of such seeds may lead to lower crop productivity and lesser yield (Ellis and Roberts 1981). This amount of deterioration in seed quality differs with plant species and seed lots, but presence of higher moisture and temperature constantly hasten this process (Goela et al. 2003; Kibinza et al. 2006). The vigor of such aged seeds could only be rejuvenated through extrinsic treatments, which are termed as invigoration techniques. Seed priming is amid those techniques and is adopted to boost up germination related characters (process, rate, uniformity and percentage) and emergence of many species (Bradford 1986). Many priming techniques have been evolved which are being utilized in many crops nowadays. Among these, hydropriming, halopriming and osmopriming are most common and popularly adopted techniques (Nawaz et al. 2013). In this particular study, the effect of hydropriming on pea seeds is investigated. Hydropriming, reported as an economical method by Harris (1992) with many advantageous effects on field crops, such as maize, rice, chickpea, soybean and sunflower (Kaya et al. 2006). Its significance has already been shown for wheat (Harris et al. 2001), chickpea (Musa et al. 2001), maize (Ashraf and Rauf 2001), sunflower (Kaya et al. 2006) and barley (Abdulrahmani et al. 2007). Research on priming has proved that seeds which are hydroprimed, tends to germinate early with root and shoot development, they grew more vigorously and germination associated traits are significantly greater than the seeds which are not primed (Mohammadi et al. 2008).

There are certain factors which are responsible for an effective hydropriming process. Optimization of such process affecting parameters is among the predominant steps which will result in the development of an efficient and complete set of standardization of all process parameters. Response surface methodology (RSM) was therefore used for optimization and is proved to be an efficient tool where many factors and their interactions affecting the response can be identified with lesser experimental trials. Box–Behnken design (BBD), a second order design based on three-level incomplete factorial design was adopted. This design does not have combinations for the process variables involved simultaneously at their highest or lowest levels. This helps in avoiding the execution of experiments under extreme conditions, for which there exists a chance for the occurrence of unsatisfactory results (Ferreira et al. 2007).

The only existing lacuna in hydropriming technique is that it is being practiced manually at laboratory scale where it is very difficult to control the process affecting parameters. Hence, there is a definite need to mechanize this process with the proper and precise control over the factors. To overcome this research gap, this study was therefore undertaken for the development and performance evaluation of a prototype which helps in hydropriming of pea seeds. The study also involves the optimization of process parameters for effective and efficient application.

Materials and methods

Materials

Pea seeds cv. *Arkel* were used for performing experiments in this particular research investigation. Two different seed lots were procured from National Seeds Corporation, New Delhi (India) and ICAR-IARI regional station Karnal (India), respectively. They were found to have 80 and 75 % of initial germination percentage, respectively. Any kind of foreign substance, such as dirt, stones, chaff and field trash along with immature/broken seeds was removed manually from the seed lots before using them for experiments.

Seed lots preparation

According to the seed standards for minimum limits for germination to maintain its physical purity, minimum germination percentage for pea seeds is 75 % (Indian Minimum Seed Certification Standards 2013). Therefore, to evaluate the comparative effect of hydropriming, seed lots were selected in such a way that among the three selected lots for the study, one lot will be of minimum germination standard, one should be below that and one should be above minimum germination percentage. Accelerated aging (40 ± 1 °C and 100 % RH) was performed on available seed lots (80 and 75 % germination) to obtain the required seed lots. As seeds are living entity, environmental factors viz. moisture content of seeds, relative humidity and temperature constitutes as the major causes for seed aging (Radha et al. 2014). Samples were withdrawn after first and second day, respectively and tested subsequently for germination as well as moisture content. The extensive results were helpful in obtaining three different seed lots with variable germination percentage and moisture content viz. L_1 (80 and 14.94 % d.b), L_2 (70 and 25.47 % d.b) and L_3 (60 and 28.04 % d.b), respectively. Initial germination of the seeds refers to germination of the seeds prior to hydropriming treatment and final germination relates with the germination of seeds subsequent to hydropriming.

Experimental design

Several advantages like adequate distribution of information across the experimental range (rotatability), minimum number of treatment combinations, good lack of fit detection, good graphical analysis are associated with RSM designs (Box and Draper 1987). The overall statistical analysis comprises design of experiments, selection of levels for process variables, fitting mathematical models, and finally selecting the optimal variable levels by either numerical/graphical optimization of the response (Khuri and Cornell 1987). Consequently, for this research study, RSM has been adopted to examine the simultaneous effect of hydropriming affecting parameters on quality traits of aged pea seeds.

Experiments were laid down with BBD considering five independent variables at three levels viz. germination percentage, soaking duration, process temperature, rotation speed and air flow rate. Range of variables was determined based on the review as well as preliminary experimental trials. Design expert (8.0.7.1) was used for optimization and for plotting the surface plots. To proceed further with RSM analysis, coding of variable levels is considered as an important step which was carried out using following equations:

$$X1 = \frac{\text{Germination percentage} - 70}{10},$$

$$X2 = \frac{\text{Soaking duration} - 45}{15},$$

$$X3 = \frac{\text{Temperature} - 25}{5},$$

$$X4 = \frac{\text{Rotation speed} - 340}{20},$$

$$X5 = \frac{\text{Air flow rate} - 0.548}{0.137},$$

where, X_1, X_2, X_3, X_4 and X_5 represents the coded values for IGP (%), SD (min), T (°C), rpm and AFR (m^3/min), respectively.

Coding of process variables brings simplification in computational procedure which further helps in bringing the same range of independent variables irrespective of their actual magnitudes. Magnitude of regression coefficients depicts the effect of process variables at linear, quadratic and interactive level. Coefficient values on the higher side showed more profound effect of that particular variable on the response (Rana et al. 2012). Coded and actual values of the processing variables are reported in Table 1. Total number of experimental trials came out to be 46. Trials were executed as per the run order (Table 2) to minimize the systematic bias in observed responses due to extraneous factors. The insignificant terms from full second order polynomial equation were excluded to get the best-fitted simple predictive equation.

Determination of process responses

Seed lots obtained after being treated with variable conditions of hydropriming were dried back for 24–48 h to their respective initial moisture levels under ambient room conditions. Subsequent to drying, seeds were subjected for estimation of quality attributes. Three replications for each dependent parameter were evaluated.

Table 1 Coded and actual levels of process variables

Independent variables	Coded and actual levels		
	−1	0	1
Germination percentage, % (X_1)	60	70	80
Priming duration, min (X_2)	45	60	75
Temperature, °C (X_3)	20	25	30
Rotation speed, rpm (X_4)	320	340	360
Air flow rate, m^3/min (X_5)	0.411	0.548	0.685

Table 2 Five factor, three-level Box–Behnken experimental design with coded and actual values (Coded values in parenthesis)

Run	IGP (X_1)	SD (X_2)	T (X_3)	rpm (X_4)	AFR (X_5)
1	70 (0)	45 (−1)	25 (0)	340 (0)	0.685 (1)
2	70 (0)	45 (−1)	25 (0)	340 (0)	0.411 (−1)
3	70 (0)	45 (−1)	25 (0)	360 (1)	0.548 (0)
4	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
5	80 (1)	45 (−1)	25 (0)	340 (0)	0.548 (0)
6	60 (−1)	75 (1)	25 (0)	340 (0)	0.548 (0)
7	60 (−1)	60 (0)	25 (0)	340 (0)	0.411 (−1)
8	70 (0)	60 (0)	25 (0)	320 (−1)	0.411 (−1)
9	70 (0)	60 (0)	30 (1)	360 (1)	0.548 (0)
10	70 (0)	60 (0)	25 (0)	360 (1)	0.685 (1)
11	80 (1)	60 (0)	30 (1)	340 (0)	0.548 (0)
12	60 (−1)	60 (0)	25 (0)	320 (−1)	0.548 (0)
13	70 (0)	60 (0)	20 (−1)	340 (0)	0.411 (−1)
14	70 (0)	60 (0)	20 (−1)	320 (−1)	0.548 (0)
15	70 (0)	75 (1)	20 (−1)	340 (0)	0.548 (0)
16	80 (1)	60 (0)	20 (−1)	340 (0)	0.548 (0)
17	80 (1)	60 (0)	25 (0)	360 (1)	0.548 (0)
18	60 (−1)	45 (−1)	25 (0)	340 (0)	0.548 (0)
19	70 (0)	60 (0)	25 (0)	360 (1)	0.411 (−1)
20	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
21	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
22	70 (0)	75 (1)	25 (0)	340 (0)	0.411 (−1)
23	70 (0)	60 (0)	30 (1)	340 (0)	0.411 (−1)
24	70 (0)	60 (0)	25 (0)	320 (−1)	0.685 (1)
25	80 (1)	75 (1)	25 (0)	340 (0)	0.548 (0)
26	70 (0)	45 (−1)	25 (0)	320 (−1)	0.548 (0)
27	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
28	70 (0)	60 (0)	30 (1)	340 (0)	0.685 (1)
29	60 (−1)	60 (0)	25 (0)	340 (0)	0.685 (1)
30	60 (−1)	60 (0)	20 (−1)	340 (0)	0.548 (0)
31	70 (0)	75 (1)	25 (0)	340 (0)	0.685 (1)
32	80 (1)	45 (0)	25 (0)	320 (−1)	0.548 (0)
33	70 (0)	75 (1)	30 (1)	340 (0)	0.548 (0)
34	60 (−1)	60 (0)	30 (1)	340 (0)	0.548 (0)
35	80 (1)	60 (0)	25 (0)	340 (0)	0.685 (1)
36	70 (0)	75 (1)	25 (0)	360 (1)	0.548 (0)
37	70 (0)	60 (0)	30 (1)	320 (−1)	0.548 (0)
38	80 (1)	60 (0)	25 (0)	340 (0)	0.411 (−1)
39	60 (−1)	60 (0)	25 (0)	360 (1)	0.548 (0)
40	70 (0)	60 (0)	20 (−1)	360 (1)	0.548 (0)
41	70 (0)	75 (1)	25 (0)	320 (−1)	0.548 (0)
42	70 (0)	45 (−1)	30 (1)	340 (0)	0.548 (0)
43	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
44	70 (0)	60 (0)	25 (0)	340 (0)	0.548 (0)
45	70 (0)	60 (0)	20 (−1)	340 (0)	0.685 (1)
46	70 (0)	45 (−1)	20 (−1)	340 (0)	0.548 (0)

Moisture content of seeds (MC)

Hydroprimed seeds which were obtained after each experimental run were subjected for moisture content determination. After the removal of surface moisture, the post hydropriming seed samples were kept immediately in hot air oven at 105 ± 1 °C for 24 h (Suthar and Das 1996).

Final germination percentage (FGP)

Germination test of hydroprimed pea seeds was conducted as per the ISTA (1993) standards. The first and second germination counts were taken on fifth and eighth day for the samples kept in germination chamber maintained at 20 °C and 90 % RH.

Seedling length (SL)

After the completion of germination test, ten random seedlings were taken for estimation of seedling length. The data was computed, averaged and expressed in centimeters.

Seedling dry weight (SDW)

Similarly, ten random seedlings were selected and were kept in hot air oven after wrapping in butter paper at 70 °C until it reaches to a constant weight. It was expressed as mg/10 seedling.

Vigor indices (VI–I and VI–II)

The estimation of final germination count, seedling length and seedling dry weight for each seed lot was linked with the estimation of vigor indices which were calculated using the method of Abdul-Baki and Anderson (1973).

Electrical conductivity (EC)

Hydroprimed seeds were subjected for electrical conductivity test where seed leachate was used for taking the observations in EC meter (Presley 1958).

Radiography

Radiography analysis was done to examine the internal seed damage which might have occurred during hydropriming. A sample of few seeds after each experimental run was subjected for X-ray treatment using Faxitron X-ray equipment available at ICAR-NBPGR, New Delhi.

Statistical analysis

The process responses attained through the proposed experimental design were subjected to regression analysis to examine the effects of independent factors. A second order model (Eq. 1) was fitted to the experimental data for all responses using least square regression analysis. Quadratic model (Khuri and Mukhopadhyay 2010) was used for obtaining regression coefficients (β_0, β_i, β_{ij} and β_{ii}) for all responses and were computed by Design Expert (8.0.7.1),

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{ii} X_i^2, \qquad (1)$$

where, X_i, X_j are the independent variables and Y-response (dependent variable), n is the number of dependent variables.

Optimization of hydropriming process variables

Optimization of process parameters was performed using numerical optimization technique in Design Expert (8.0.7.1) by setting up desired goals for both independent and dependent parameters. Optimal solution having maximum desirability has been selected and as a part of validation, trials were carried out for further reassurance of those process conditions.

Results and discussion

Quality attributes of the hydroprimed seeds were determined using the standard experimental procedure. The estimated regression coefficients and ANOVA related with the second order polynomial models for process responses are reported in Table 3. Model analysis revealed that regression models for

Table 3 ANOVA and regression coefficients of the second order polynomial models for response variables

Parameters	MC (% d.b)	FGP (%)	SL (cm)	SDW (mg)	VI–I	VI–II	EC (mS/cm)
Model	42.935	75.833	23.883	334.017	1812.102	25,332.417	0.275
X_1	−1.548**	9.875***	1.767***	8.029*	323.313***	3407.698***	−0.061***
X_2	4.247***	−1.125**	−0.214	−12.351**	−32.104	−1097.977**	0.017***
X_3	0.871	−1.063*	−0.864*	1.334	−92.273**	−293.511	0.020***
X_4	1.681***	−4.375***	−2.037***	−22.701***	−230.668***	−2877.54***	0.023***
X_5	0.614	0.188	0.308	3.209	25.999	269.457	0.002
X_1 X_2	2.576**	2.750**	0.165	17.893*	52.223	1738.4*	−0.019
X_1 X_3	0.345	0.750	0.035	1.613	6.623	264.740	−0.001
X_1 X_4	0.063	−0.250	−0.038	8.443	−28.620	281.793	0.000
X_1 X_5	−0.343	−0.250	0.105	−2.060	2.593	−283.930	0.002
X_2 X_3	0.126	2.250**	2.388**	16.563*	211.210**	1811.773*	0.021*
X_2 X_4	3.235**	−1.750	−1.365	−13.945	−129.083	−1438.240	−0.017
X_2 X_5	0.089	0.250	−0.213	−0.305	−9.662	60.065	−0.008
X_3 X_4	−0.110	1.000	0.440	2.380	51.840	428.683	−0.001
X_3 X_5	−0.177	0.750	−0.343	1.593	−9.385	357.580	−0.04***
X_4 X_5	0.923	0.000	0.130	16.025	4.373	1093.343	0.001
X_1^2	−4.234***	−1.208	−2.345***	−31.627***	−184.988***	−2600.30***	−0.014*
X_2^2	−0.448	−3.542***	−2.329***	−15.825**	−241.225***	−2157.02***	−0.008
X_3^2	−0.195	−0.292	−1.092*	−4.097	−83.097	−377.05	−0.025***
X_4^2	−0.179	−3.708***	−2.620***	−23.429***	−264.286***	−2793.46***	0.007
X_5^2	−0.140	0.042	−0.902	−1.799	−71.778	−239.825	0.000
ANOVA							
R^2	0.8212	0.9504	0.7765	0.7729	0.8527	0.8557	0.8762
Model (F value)	5.74***	23.95***	4.34***	4.25***	7.23***	7.41***	8.85***
Lack of fit (P value)	0.0047	0.0672	0.1109	0.0004	0.1350	0.0075	0.8385
C.V. %	5.64	2.94	8.46	6.01	10.86	8.24	9.06

X_1 coded germination percentage level, *X_2* coded soaking duration, *X_3* coded temperature; *X_4* coded rotation speed, *X_5* coded air flow rate, *MC* moisture content, *FGP* final germination percentage, *SL* seedling length, *SDW* seedling dry weight, *VI–I* vigor index-I, *VI–II* vigor index-II, *EC* electrical conductivity, *CV* coefficient of variation

* Significant at 10 % ($P < 0.1$)

** Significant at 5 % ($P < 0.05$)

*** Significant at 1 % ($P < 0.01$)

moisture content (MC), final germination percentage (FGP), seedling length (SL), seedling dry weight (SDW), vigor indices (VI–I and VI–II) and electrical conductivity (EC) were highly significant ($P \leq 0.01$), with coefficient of determination values ($R^2 \geq 0.77$). This proves that the developed models had the capability of being used to navigate the design space and envisage the responses in accurate manner. Any particular coefficient will be highly significant, if the corresponding '*p*' value is smaller (Kalil et al. 2000). Representation of F values showed that all the developed models (MC, FGP, SL, SDW, VI–I, VI–II and EC) were significant. Regression coefficients with positive values indicate synergetic effect, while antagonistic effect was indicated by negative values.

Moisture content after hydropriming (MC)

Moisture content after hydropriming of the seeds is a measure of their water potential. Seed lots subjected to variable conditions of hydropriming tend to absorb moisture during the process, and it was found that moisture uptake by accelerated aged seed lots was more as compared to fresh seeds. Moisture content was found in range of 30.32–51.78 % d.b. Linear effects of variables viz. initial germination percentage ($P \leq 0.05$), soaking duration and rotation speed ($P \leq 0.01$), interaction effects of initial germination and soaking duration, soaking duration and rotation speed ($P \leq 0.05$) and quadratic effect of initial germination percentage ($P \leq 0.01$) were the key influential factors for moisture content of pea seeds (Table 3).

Initial germination percentage of the seeds had negative linear effect, i.e., moisture absorption capability of low vigorous seeds was found to be more as compared to the fresh seeds. It must be due to the reason that cellular membrane of seed lots which were exposed to accelerated aging becomes more permeable, and thereby absorbing more water during priming process compared with unaged seeds. Response surface plot (Fig. 1) also substantiated the reduction of moisture absorption capability of seeds with the increase in initial germination percentage, whereas positive linear trend was illustrated with respect to soaking duration as well as rotation speed. This positive relationship might be due to the absorption of more moisture with prolonged soaking and because of the increasing higher speed; uniform moisture uptake by the seeds will take place along its surface. R^2 value (0.82) of the model indicated that the model adequately represented the relationships among the selected process variables. The overall second order polynomial equation for moisture content can be written as follows (Eq. 2):

$$\begin{aligned}\mathrm{MC} &= 42.93 - 1.55X_1 + 4.15X_2 + 1.68X_4 + 2.58X_1X_2 \\ &\quad + 3.23X_2X_4 - 4.13X_1^2 \end{aligned} \tag{2}$$

Final germination percentage (FGP)

Germination test which has been certified due to its reproducibility was used to evaluate seed quality (Perry 1981). It is one of the important parameter which depicts the success/failure of the hydropriming treatment given to aged seed lots. Linear increasing trend in FGP was observed with soaking duration and rotation speed up to a certain limit which was further decreased at their corresponding higher values. Regression coefficient (Table 3) revealed positive linear significant effect ($P \leq 0.01$) of initial germination, while the linear effect of rotation speed ($P \leq 0.01$), soaking duration ($P \leq 0.05$) and temperature ($P \leq 0.01$) were negative. Interaction effect of both (initial germination × soaking time) along with (soaking

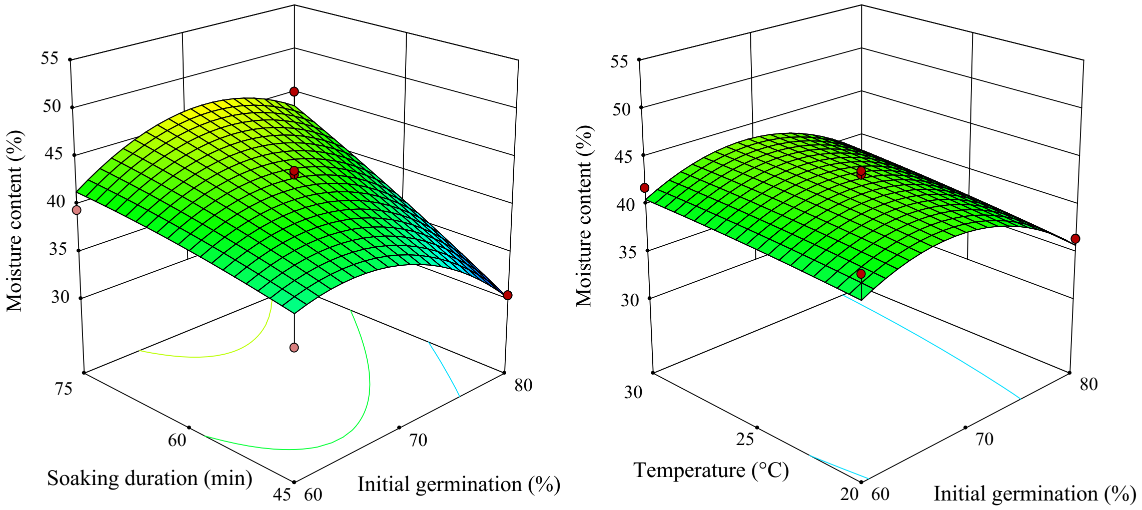


Fig. 1 Response surface plot for moisture content as a function of initial germination percentage, soaking duration and temperature

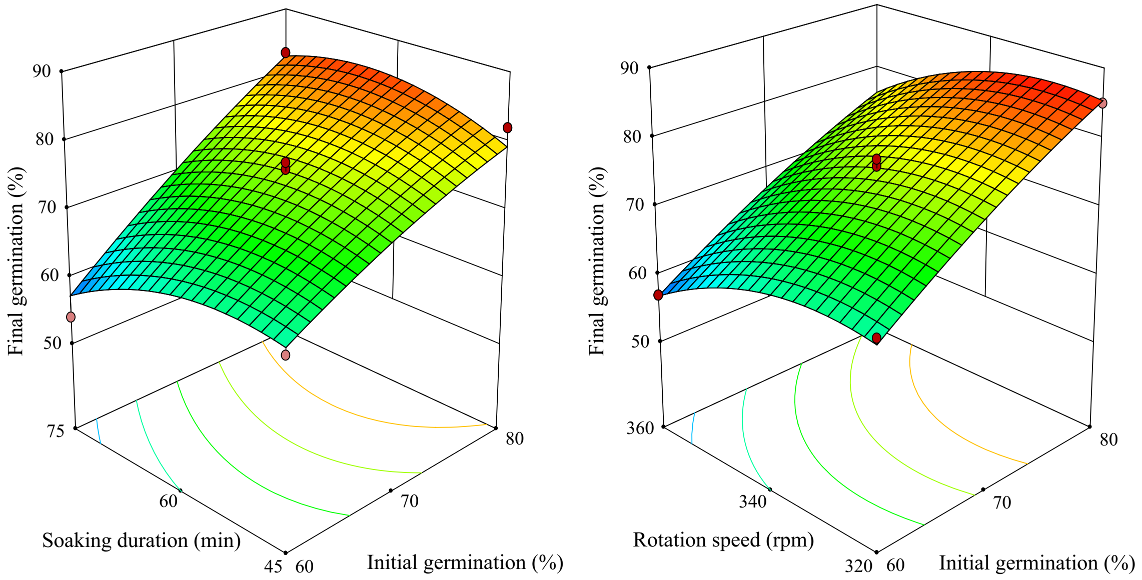


Fig. 2 Response surface plot for final germination as a function of initial germination percentage, soaking duration and rotation speed

time × temperature) at 5 % level of significance and quadratic effect of soaking duration as well as rotation speed were significant at 1 % level of significance, respectively. Coefficient of determination ($R^2 = 0.95$) suggesting that 95 % variability in the data was explained by the model. Response surface plots (Fig. 2) showed increase in FGP as compared to corresponding initial germination, however, the percentage increase in final germination after hydropriming was more in low vigorous seeds, i.e., (60 and 75 % seed lots) as compared with highly vigorous seeds (80 % seed lot). This was attributed to the positive response of low vigorous seeds to hydropriming rather than high vigor seeds. The possible reason for early and enhanced germination of primed seed lies in the fact that there is completion of pre-germinative metabolic processes which gives a primed seeds a head start over the un-primed seed making the seed ready for radicle protrusion (Varier et al. 2010). Germination enhancement can also be attributed to metabolic repair processes during process of seed priming (Mehta et al. 2010).

Another pertinent reason for the decrease of FGP at higher values of soaking time and rotation speed was attributed to the presence of thin seed coat of pea seeds which got gradually damaged with prolonged soaking and at higher rotation speed. This further resulted in the increase of more number of dead and abnormal seeds after completion of experimental run. Empirical relationship between FGP and the process variables in coded form was represented by following regression equation (Eq. 3):

$$\begin{aligned}\mathrm{FGP} &= 75.83 - 1.12X_1 - 1.06X_2 + 0.87X_3 - 4.37X_4 \\ &\quad + 2.75X_1X_2 + 2.25X_2X_3 - 3.54X_2^2 - 3.71X_4^2\end{aligned} \tag{3}$$

Seedling length (SL)

SL for pea seed lots ranged from 15.83 to 26.71 cm throughout the experimental run. Linear effects of initial germination and rotation speed were more significant ($P \leq 0.01$) as compared to those of process temperature ($P \leq 0.1$) as evident from Table 3. However, the effect of initial germination was positive, while that of rotation speed was negative. Also, interaction effect of soaking duration and process temperature was significant ($P \leq 0.05$). Parameters viz. initial germination, soaking duration and rotation speed were observed to have higher effect ($P \leq 0.01$) along with effect of temperature ($P \leq 0.05$) in their respective quadratic terms. Coefficient of determination (R^2) of the model was 0.77 (Fig. 3).

Declination of SL with both soaking time and rotation speed was observed and the pertaining reason would be the reduction in final germination of seeds exposed to longer priming duration and higher rotation speed during hydropriming (Fig. 4). Damage of thin seed coat of pea seeds supposed to be the major reason for the reduction in seed quality parameters. Significant terms related to SL can be written as follows (Eq. 4):

$$\begin{aligned}\mathrm{SL} &= 23.88 + 1.71X_1 - 0.86X_3 - 2.04X_4 + 2.39X_2X_3 \\ &\quad - 2.34X_1^2 - 2.33X_2^2 - 1.09X_3^2 - 2.62X_4^2\end{aligned} \tag{4}$$

Seedling dry weight (SDW)

SDW ranged from 223.54 to 348.56 mg/10 seedlings for pea seed lots. The effect of rotation speed in its linear term was negative ($P \leq 0.01$), while the linear effects of

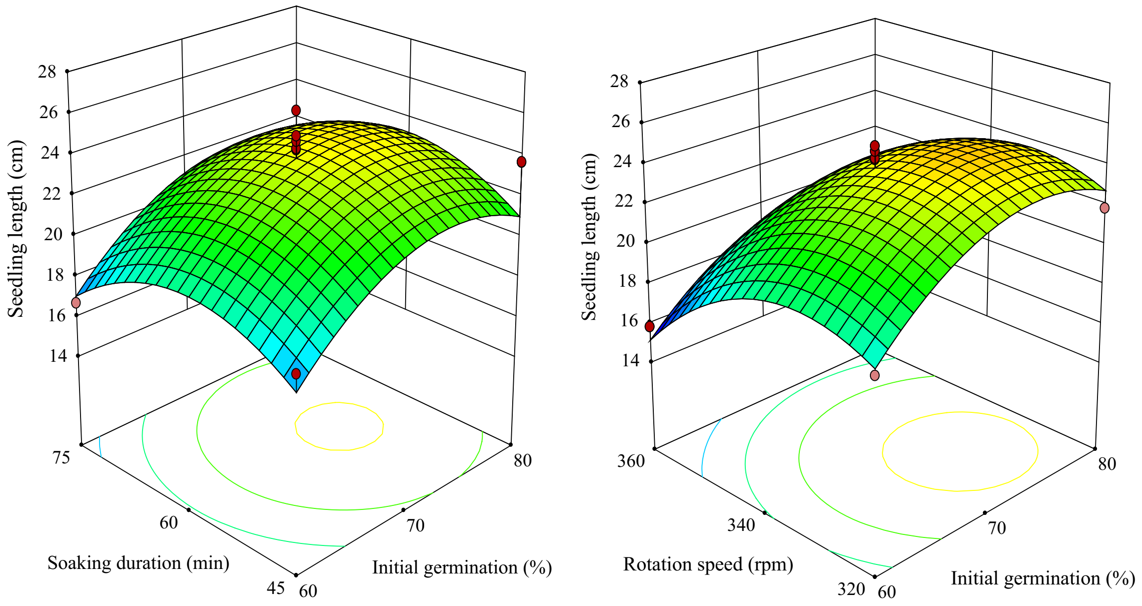


Fig. 3 Response surface plot for seedling length as a function of initial germination percentage, soaking duration and rotation speed

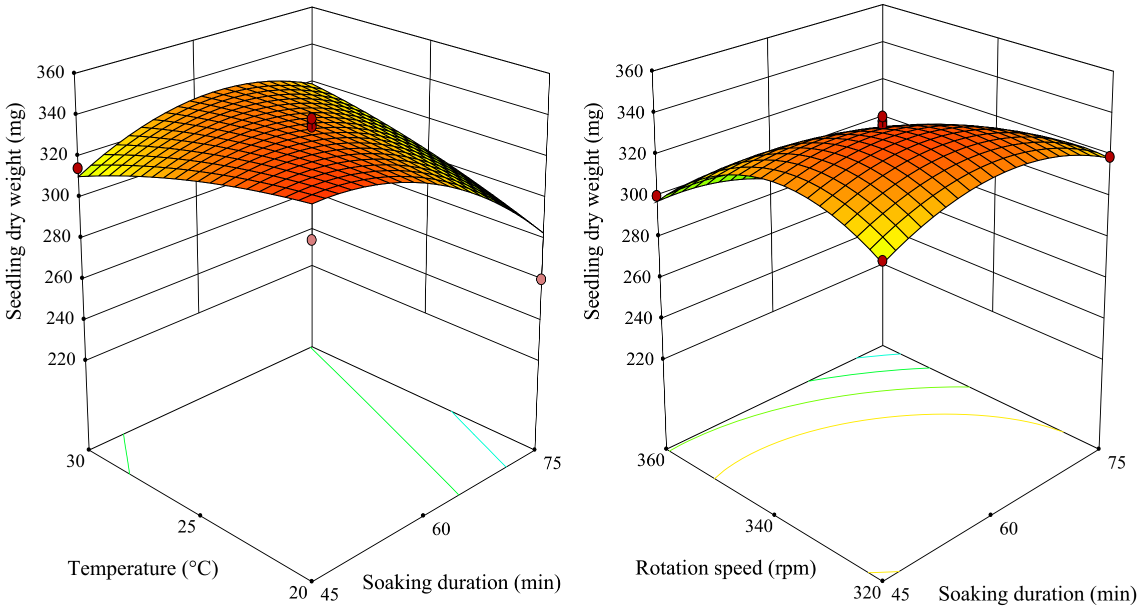


Fig. 4 Response surface plot for seedling dry weight as a function of soaking duration, temperature and rotation speed

soaking duration ($P \leq 0.05$) and initial germination ($P \leq 0.1$) were positive. Interaction effects (initial germination × soaking duration) and (soaking duration × temperature) were also effective at $P \leq 0.1$. Also the respective quadratic terms of parameters viz. initial germination, rotation speed collectively had their significant effect ($P \leq 0.01$) while soaking duration was found affecting SDW at 5 % level of significance.

Figure 4 showed SDW followed a decreasing trend at higher values of soaking time, temperature and rotation speed which was a possible result of diminishing values of FGP of seed lots. The obvious reason was increase in number of dead seeds due to the constant damage to seed coat of pea seeds. Second order polynomial equation for SDW can be written as follows (Eq. 5):

$$\begin{aligned}\mathrm{SDW} &= 334.02 + 8.03X_1 - 12.35X_2 - 22.70X_4 \\ &\quad + 17.89X_1X_2 + 16.56X_2X_3 - 31.63X_1^2 - 15.82X_2^2 \\ &\quad - 23.43X_4^2 \qquad (5)\end{aligned}$$

Vigor indices (VI–I and VI–II)

Vigor indices are an alternative way of representing the combined consequences of germination values post hydropriming, seedling length as well as seedling dry weight. R^2 values for both VI–I and VI–II were 0.85 and 0.85, respectively. Hydroprimed pea seed lots exhibited VI–I varying from 902.31 to 2083.38. Regression result (Table 3) depicted the linear dependence of initial germination on VI–I. Both process temperature and rotation speed were found to be negatively correlated with VI–I and rotation speed effect was more prominent supported by its lower regression coefficient. The curve moving downwards revealed reduction in VI–I at higher rotation speed (Fig. 5). Interaction effect of soaking time and temperature also found affecting VI–I ($P \leq 0.05$). Quadratic effects of initial germination, soaking duration and rotation speed were negatively correlated with VI–I ($P \leq 0.01$). Result of regression analysis in the form of polynomial equation can be expressed as follows (Eq. 6):

$$\begin{aligned}\text{VI-I} &= 1812.10 + 323.31X_1 - 92.27X_3 - 230.67X_4 \\ &\quad + 211.21X_2X_3 - 184.99X_1^2 - 241.22X_2^2 \\ &\quad - 264.29X_4^2 \end{aligned} \tag{6}$$

Values of VI–II for hydroprimed pea seed lots were from 12,071.2 to 27440. Regression analysis (Table 3) showed initial germination ($P \leq 0.01$), rotation speed ($P \leq 0.01$) and soaking time ($P \leq 0.05$) were highly significant at their linear as well as quadratic levels, respectively. Interaction effects (initial germination × soaking time) along with (soaking time × temperature) were also found affecting VI–II ($P \leq 0.1$). Curved surface plot (Fig. 6) showed an initial increase up to a certain limit followed by a significant reduction with soaking time, temperature and rotation speed. Second order polynomial equation for VI–II can be written as follows (Eq. 7):

$$\begin{aligned}\text{VI-II} &= 25332.42 + 3407.70X_1 - 1097.98X_2 \\ &\quad - 2877.54X_4 + 1738.40X_1X_2 + 1811.77X_2X_3 \\ &\quad - 2600.3X_1^2 - 2157.02X_2^2 - 2793.46X_4^2 \end{aligned} \tag{7}$$

Vigor indices were amplified up to a certain limit with both soaking time and initial germination but afterwards it followed a decreasing trend. Response curve also revealed reduction in vigor indices at higher rotation speed which was due to the transformation of normal seeds into dead ones because of the damage to their seed coat during the experimental process. The experimental results were found to be in agreement with Rudrapal and Naukamura (1988), who reported the increased development of stronger and efficient root and shoot system as well as vigor index by hydropriming in egg plant and radish. Similar results have been reported by Stofella et al. (1992) in osmoprimed (PEG) seeds of bell pepper (*Capsicum annum*).

Electrical conductivity (EC)

EC correlates with the negativity aspects of seed quality (Takayanagi and Murakami 1968). EC values ranged from 0.172 to 0.346 mS/cm for pea seed lots. There was highly significant effect ($P \leq 0.01$) of initial germination, soaking duration, process temperature and rotation speed at their respective linear levels. However, the influence of initial germination was negative as can be

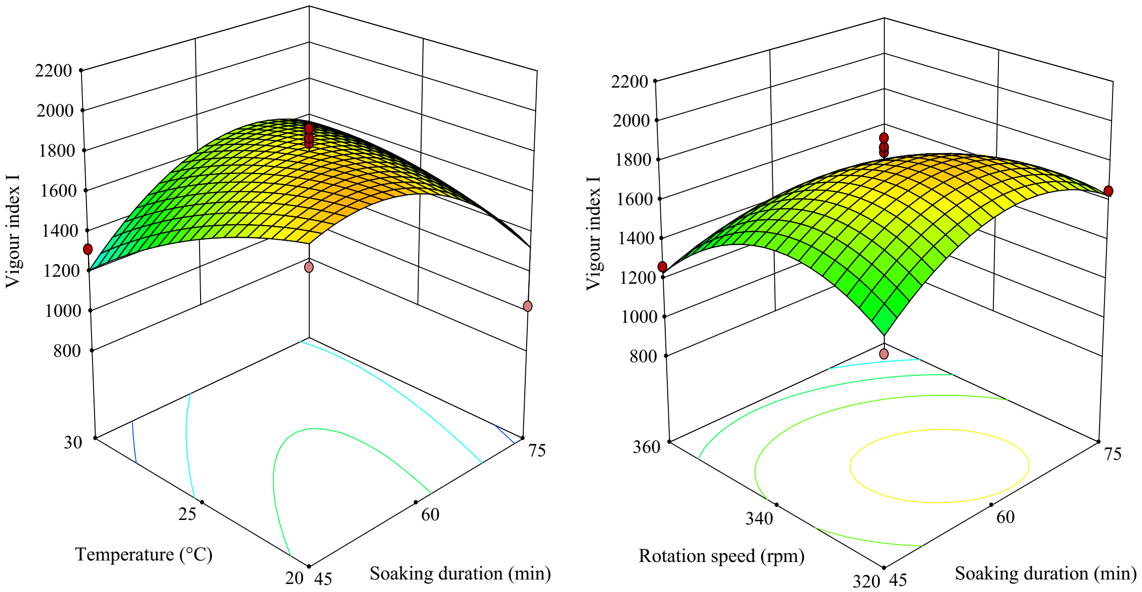


Fig. 5 Response surface plot for VI–I as a function of soaking duration, temperature and rotation speed

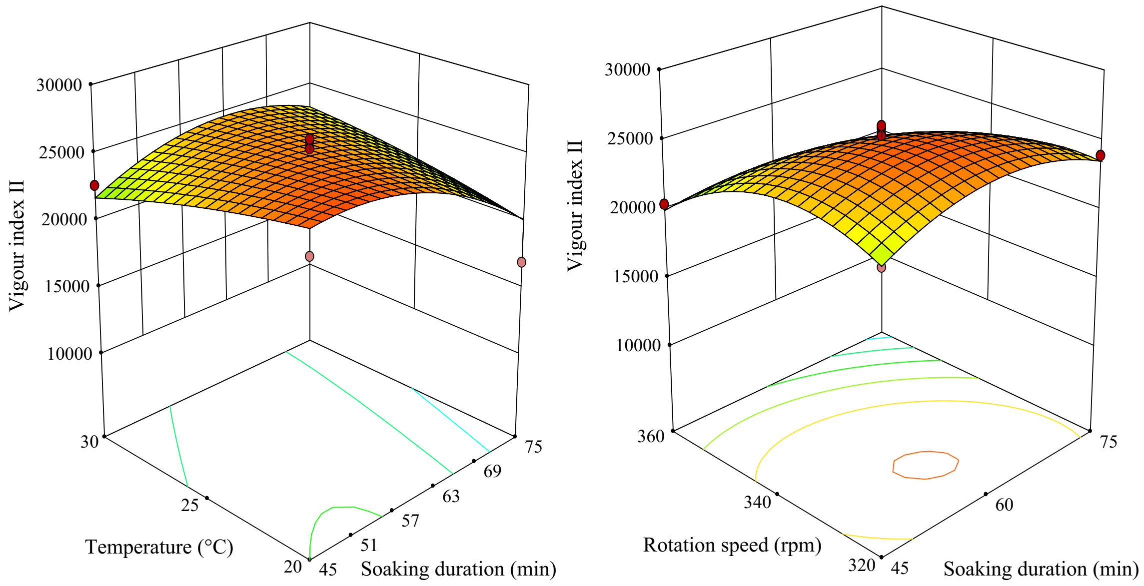


Fig. 6 Response surface plot for VI–II as a function of soaking duration, temperature and rotation speed

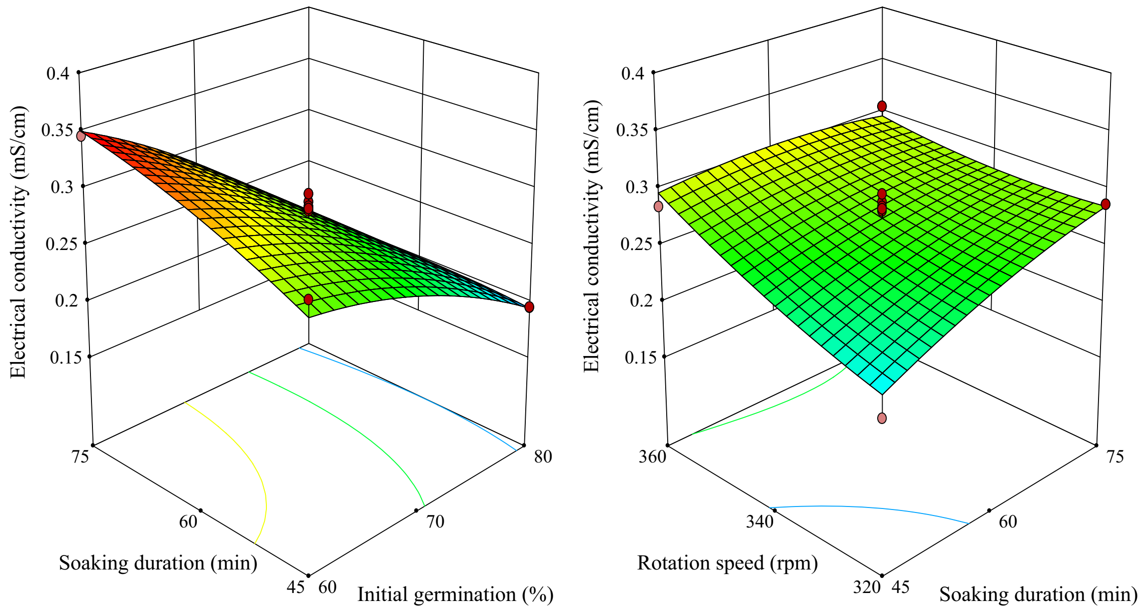


Fig. 7 Response surface plot for EC as a function initial germination, soaking duration and rotation speed

observed from its regression coefficient (Table 3). Also, there was negative interaction effect of temperature and air flow rate which was found significant ($P \leq 0.01$), while the combined effect of soaking duration with temperature was positive in lower terms ($P \leq 0.1$). Reduction in EC values with increase in germination of seeds was observed, but found rising with soaking time, temperature and rotation speed (Fig. 7). Regression equation (Eq. 8), which was an empirical relationship of EC after hydropriming and the test variables in coded form was:

$$\begin{aligned}\mathrm{EC} &= 0.28 - 0.061X_1 + 0.017X_2 + 0.02X_3 + 0.023X_4 \\ &\quad + 0.021X_2X_3 - 0.04X_3X_5 + 7.5\mathrm{E}-004X_4X_5 \\ &\quad - 0.014X_1^2 - 0.025X_3^2 \end{aligned} \tag{8}$$

This particular variation noticed in both high and low vigor seeds existed may be due to the fact that seeds which are old and having less vigor, leaches more electrolytes due to cellular membrane deterioration. Reformation of cellular membrane occurred due to priming which further reduces solute leakage in hydroprimed seeds of French bean (Pandey

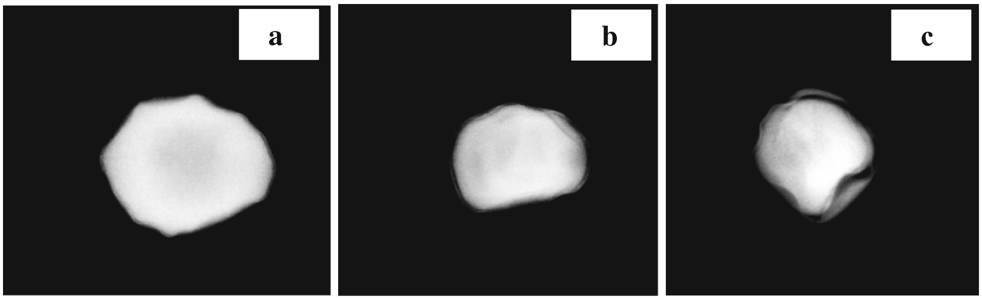
1989) and eggplant and radish (Rudrapal and Naukamura 1988). Similar kind of variation was noticed by Yogalakshmi et al. (1996) in rice.

Radiography

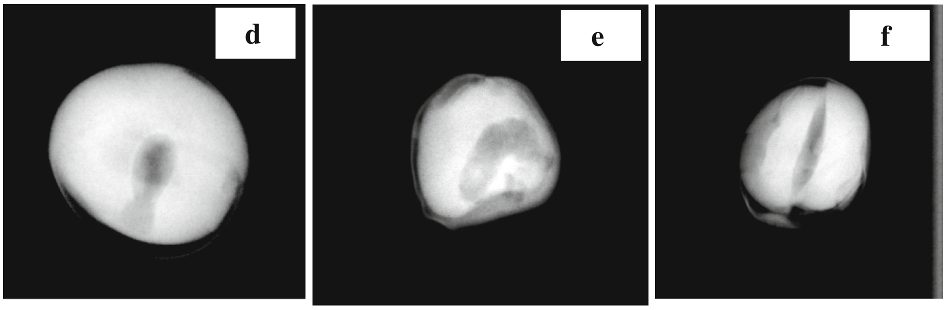
X-ray analysis of internal seed structure provides a technique to evaluate seed quality (Simak 1991). The analysis is nondestructive and allows the morphological evaluation of the embryo and endosperm in the intact seed. Radiography imaging was quite helpful to visualize the structural arrangement of seed's inner membrane. Such changes involve swelling of seeds and loosening of inner intact layers due to moisture absorption. Radiography was performed by subjecting the hydroprimed seed sample of all 46 experiments to X-ray imaging. It reveals that longer soaking duration and higher rotation speed resulted in considerable damage of seed coat. As the seed coat in pea seeds is relatively very slender, they were more prone to injury after completion of hydropriming. Radiographic view of a single seed with respect to the three seed lots prior to hydropriming, i.e., (L_1, L_2, L_3) as well as seeds subjected to variable duration of hydropriming, i.e., (45, 60 and 75 min) is captured and presented with detailed description in Fig. 8.

Optimization of process parameters

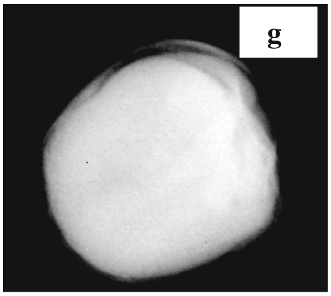
Numerical optimization was done for obtaining best suitable process conditions for hydropriming of pea seed lots using Design expert (8.0.7.1) of the STATEASE program.



80% germination seed (L_1) **70% germination seed (L_2)** **60% germination seed (L_3)**



45 min hydropriming **60 min hydropriming** **75 min hydropriming**



Optimal condition

Fig. 8 Radiography view of pea seeds after hydropriming classified as: **a** L_1 (80 % germination); **b** L_2 (70 % germination); **c** L_3 (60 % germination); **d** seed after 45 min of hydropriming showing seed coat damage and less air cavity; **e** seed after 60 min of hydropriming with more ruptured seed coat and noticeable air cavity; **f** seed after 75 min of hydropriming showing prominent presence of visible fissures in seed coat and detached cotyledons; **g** hydroprimed seed treated at optimal condition with minimal air cavity and seed coat damage

Maximum desirability of the optimized solution was obtained by assigning the desired goals for each variable and response. Goals assigned were: final moisture content kept in range, maximization of final germination, seedling length, seedling dry weight, vigor indices and minimization of electrical conductivity. Ten optimum conditions of independent variables along with the predicted values of responses were generated by the software. The particular solution having maximum desirability value was selected as optimum for hydropriming of pea seeds using seed priming prototype for obtaining maximum quality attributes. The optimum solution considered was initial germination 75 %, soaking duration 55 min, temperature 20 °C, rotation speed 320 rpm and air flow rate 0.5 m^3/min. This solution was validated experimentally for reassurance and was verified accordingly.

Conclusion

The effort made to mechanize hydropriming process using developed priming prototype for pea seeds was found to be satisfactory. Outcome of the performance evaluation proved that the developed prototype was worth adopted for hydropriming of fresh as well as aged seeds. Hydropriming treatment was proved to be substantially effective in improving the quality attributes of the subjected pea seeds through extensive trials carried out on laboratory scale. Statistical interpretation (F value, R^2, CV, lack of fit) also revealed that the models pertaining to dependent parameters were significant. Overall attempt made for the precise control of process affecting parameters was successful. Optimal solution obtained using RSM involving independent parameters was found appropriate and got validated by performing trials on those conditions. The developed prototype can further be utilized for on-farming purpose if used judiciously. However, the possibility of adopting other priming methods for different vegetable seeds using the prototype needs to be explored.

Author contribution statement Manoj Kumar Mahawar: Fabrication of the prototype, carried out experiments, data observation, formulation of the manuscript, statistical analysis of the data. DVK Samuel: Setting the objectives, selection of independent and dependent parameters, drafting the article. JP Sinha: Conception and design of the prototype, revision of the manuscript, optimization and validation of the process parameters. Kirti Jalgaonkar: Assisted in the critical editing and improving the manuscript pertaining to various steps of revision.

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