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FULL-LENGTH RESEARCH ARTICLE

Optimization of Hydropriming of Okra (*Abelmoschus esculentus*) Seeds Using Response Surface Methodology

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Abstract This research study was envisaged to evaluate the effect of parameters, viz. initial germination percentage (IGP), soaking duration (SD), temperature, rotation speed (rpm) and air flow rate (AFR) for hydropriming of Okra (*Abelmoschus esculentus* L.) seeds. Three different seed lots based on their moisture content and germination percentage were chosen in this study. Moisture content and germination percentage of the selected lots varied from 11.33 to 16.89% (d.b.) and 50 to 80%, respectively. Accelerated ageing was performed at 40 ± 1 °C, 100% relative humidity to obtain required lots from available seed sample. Response surface methodology was adopted using a five factor three-level Box–Behnken design with soaking duration (4, 5 and 6 h), temperature (20, 25, 30 °C), rotation speed, (320, 340, 360 rpm), air flow rate, (2.192, 2.740, 3.288 m^3/min) as the main process parameters. The effect of process affecting parameters was observed on responses, viz. moisture content, germination percentage, seedling length, seedling dry weight, vigour index-I, vigour index-II, electrical conductivity and radiography. Models were found to be significant for all the process responses with the optimal solution as 73% (IGP), 6 h (SD), 23 °C temperature, 330 (rpm) and 2.50 m^3/min (AFR).

Keywords Hydropriming · Okra · Accelerated aging · Optimization · Seed priming prototype

Introduction

Okra (*Abelmoschus esculentus* L.) of *Malvaceae* family is one of the important vegetable crops grown throughout India including arid and semi-arid environments where water needed for germination and emergence is available only for a short period. The successful crop establishment depends on rapid and uniform germination of the seed under low water availability [26]. Okra seeds are sown in early April in plain areas and in last week of April at higher elevations. It does not germinate below 20 °C [25]. The slow and uneven germination of okra seed restricts the early spring planting [24]. The seed germination percentage is relatively low due to occurrence of hard-seededness [7] and as seed reaches towards maturation, its moisture content decreases and fraction of hardseededness increases.

Particularly in vegetable crops, the marketable yield and maturity time are influenced by the time taken for seedling emergence. Rapid and uniform seedling emergence is a prerequisite to better stand establishment and subsequent plant growth and yield in vegetable crops [13]. During storage, seeds start deteriorating wherein vigour is the first component of seed quality which is lost, followed by a loss of germination capacity and viability. Sowing of such deteriorated seeds may result in field problems like decrease in germination percentage, heterogeneous emergence of seeds, unbalanced seedling growth and competition for environmental resources such as light, nutrients and water. To overcome this, invigouration techniques such as seed priming are followed which involves physiological enhancement of seed performance. Priming is defined as a pre-sowing treatment involving controlled

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hydration of seeds which is sufficient to allow pre-germinative metabolic activation to take place, but insufficient to allow radicle protrusion through the seed coat [23]. Asgedom and Becker [3] stated that seed priming triggers few or all pre-germination processes, which persist in the seed even after re-drying leading to quick re-imbibition and rapid revival of germination metabolism. Consequently, primed seeds are equipped with advanced germination and exhibit improved germination rate and uniformity.

Hydropriming, as proposed by Harris [11], is a low-cost method with advantageous effects for wheat [9], maize [2] and pea [19]. Factors such as crop species, water potential, priming method, priming duration, temperature, air flow rate and light will determine the success of priming in any crop [29]. The available literature reports suggested that hydropriming is performed manually at laboratory scale which consumes time as well as it becomes difficult to precisely regulate the process affecting parameters. Optimization of such parameters helps in improving the overall efficiency of hydropriming process of any seed crop. The hydropriming in this study was carried out using the developed prototype and the detailed specifications along with working mechanism were reported [20]. In concurrence of the above mentioned facts, an attempt was made to mechanize the hydropriming of okra seeds using the developed prototype and simultaneously optimization of process variables through response surface methodology.

Materials and Methods

Materials

Okra seeds of *Pusa A-4* variety were procured from National Seed Corporation, New Delhi (India), and were used for performing experiments. Seeds were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff as well as immature and broken seeds.

Seed Lot Preparation

The minimum germination limits for okra seeds to maintain its physical purity with respect to its germination percentage is 65% [14]. The intended aim of seed lot preparation was to prepare the lots in such a way that among the three selected lots, one lot was of minimum germination standard and one lot each of below and above of minimum germination percentage. This lot selection will help in understanding the effect of hydropriming in a better way. To achieve this difference in germination percentage, the available seed lots were subjected to accelerated ageing (40 °C, 100% RH) for 12 days. A test sample of few seeds was withdrawn after 5th, 8th and 12th day, respectively, and was evaluated for germination as well as moisture content. The collective results were helpful in obtaining seed lots having variation in germination percentage and moisture content, viz. L_1 (80 and 10.98% d.b.), L_2 (65 and 13.97% d.b.) and L_3 (50 and 16.89% d.b.), respectively.

Experimental Design

In this study, response surface methodology (RSM) was employed for experimental design, fitting of mathematical models, generation of 3D response surface plots and optimization of process variables as well as responses. Experimental runs were obtained using Box–Behnken design (BBD) by adopting Design Expert software (version 8.0.7.1). BBD is second-order design based on three-level incomplete factorial design and has the advantage that it does not contain combinations of all factors at their highest or lowest levels; thereby, it is useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results might occur [8]. In the present study BBD was used to design the experiments comprising five independent processing variables (initial germination percentage (IGP), soaking duration (SD), temperature (T), rotation speed (rpm) and air flow rate (AFR)) at three levels. Coded and actual levels of the independent variables are given in Table 1. The design resulted in overall of 46 experiments (Table 2), with centre point experiments were repeated six times to calculate the repeatability of the method [22].

Determination of Process Responses

Moisture Content of Seeds (After priming)

Moisture content of the seeds was determined by oven drying method at 105 ± 1 °C for 24 h [28]. Samples of the primed seeds were kept immediately for moisture content determination after experiment to avoid any experimental error.

Final Germination Percentage (FGP)

Seeds were allowed to dry in normal room condition for 24 h after completion of priming process so as to remove the surface moisture. Thereafter, germination test was conducted according to ISTA [15].

Seedling Length (SL)

Ten normal seedlings of each run were randomly selected from the germination test for measuring the seedling length. Average length of ten seedlings was measured

Table 1 Coded and actual levels of process variables

Independent variables	Coded and actual levels		
	− 1	0	1
Initial germination percentage, % (X_1)	50	65	80
Soaking duration, h (X_2)	4	5	6
Temperature, °C (X_3)	20	25	30
Rotation speed, rpm (X_4)	320	340	360
Air flow rate, m^3/min (X_5)	2.192	2.740	3.288

using a scale (least count: 1 mm) and expressed in centimetres.

Seedling Dry Weight (SDW)

Randomly taken ten normal seedlings were used for recording seedling dry weight measurement. Cotyledons were removed from the epicotyls and placed in a butter paper bag for 24 h at 70 ± 1 °C. The dried seedlings were removed and cooled in a desiccator for 30 min, then dry weight was recorded in an electronic balance, and average weight was expressed in milligram per ten seedlings.

Vigour Indices (VI-I & II)

After the final count during germination test the length and dry weight of ten randomly selected normal seedlings were measured. The vigour indices were computed as suggested by Abdul-Baki and Anderson [1].

Electrical Conductivity (EC)

A sample of 50 primed seeds was dipped in 250 ml of distilled water for 24 h at 20 °C. The flasks were stirred to remove air bubbles and floating seed, covered with aluminium foil to avoid air circulation. After soaking, seeds were gently swirled and the conductivity of seed leachate was measured with a dip type cell conductivity metre (ELICO) having cell constant of 1.0. The estimations were done in four replications, and mean value was expressed in mS/cm of seed [15].

Radiography

Soft X-ray machine 'SOFTEX- MODEL- ISTV-25' was used to inspect the internal structure of seeds after priming. It was equipped with a 12" TV monitor that receives the image of the seed through an X-ray camera. Sample of 20 seeds after each experiment was subjected for radiography to examine the seed damage.

Statistical Analysis

Process responses (MC, FGP, SL, SDW, VI-I, VI-II and EC) obtained as a result of the proposed experimental design were subjected to regression analysis in order to assess the effects of independent factors. A second-order model (Eq. 1) was fitted to the experimental data for each response using least square regression analysis. Quadratic model [18] was used for obtaining regression coefficients (β_o, β_i, β_{ij} and β_{ii}) for all responses and was computed by Design Expert (version 8.0.7.1) software.

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{ii} X_1^2 \qquad (1)$$

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

Optimization of Priming Process Variables

The optimum values of process parameters were obtained using numerical optimization technique of Design Expert (version 8.0.7.1). The software necessitates assigning goals to the variables and the responses. All the variables were kept within range, while the responses were either maximized (FGP, SL, SDW, VI-I, VI-II), minimized (EC) or kept in range (MC). Optimal solution having maximum desirability has been selected, and experiments were carried out on those conditions to reassure those values [4].

Results and Discussion

Hydroprimed seeds were evaluated for their quality parameters by following the standard methodologies. Estimated regression coefficients of second-order polynomial models for the responses and their statistical validity defining values are reported in Table 3. The model coefficients for MC, FGP, SL, SDW, VI-I, VI-II and EC were highly significant ($P \leq 0.01$), with a relatively higher

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

Run	IGP	SD	Temp	rpm	AFR
1	65 (0)	4 (− 1)	25 (0)	340 (0)	3.288 (1)
2	65 (0)	4 (− 1)	25 (0)	340 (0)	2.192 (− 1)
3	65 (0)	4 (− 1)	25 (0)	360 (1)	2.740 (0)
4	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
5	80 (1)	4 (− 1)	25 (0)	340 (0)	2.740 (0)
6	50 (− 1)	6 (1)	25 (0)	340 (0)	2.740 (0)
7	50 (− 1)	5 (0)	25 (0)	340 (0)	2.192 (− 1)
8	65 (0)	5 (0)	25 (0)	320 (− 1)	2.192 (− 1)
9	65 (0)	5 (0)	30 (1)	360 (1)	2.740 (0)
10	65 (0)	5 (0)	25 (0)	360 (1)	3.288 (1)
11	80 (1)	5 (0)	30 (1)	340 (0)	2.740 (0)
12	50 (− 1)	5 (0)	25 (0)	320 (− 1)	2.740 (0)
13	65 (0)	5 (0)	20 (− 1)	340 (0)	2.192 (− 1)
14	65 (0)	5 (0)	20 (− 1)	320 (− 1)	2.740 (0)
15	65 (0)	6 (1)	20 (− 1)	340 (0)	2.740 (0)
16	80 (1)	5 (0)	20 (− 1)	340 (0)	2.740 (0)
17	80 (1)	5 (0)	25 (0)	360 (1)	2.740 (0)
18	50 (− 1)	4 (− 1)	25 (0)	340 (0)	2.740 (0)
19	65 (0)	5 (0)	25 (0)	360 (1)	2.192 (− 1)
20	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
21	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
22	65 (0)	6 (1)	25 (0)	340 (0)	2.192 (− 1)
23	65 (0)	5 (0)	30 (1)	340 (0)	2.192 (− 1)
24	65 (0)	5 (0)	25 (0)	320 (− 1)	3.288 (1)
25	80 (1)	6 (1)	25 (0)	340 (0)	2.740 (0)
26	65 (0)	4 (− 1)	25 (0)	320 (− 1)	2.740 (0)
27	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
28	65 (0)	5 (0)	30 (1)	340 (0)	3.288 (1)
29	50 (− 1)	5 (0)	25 (0)	340 (0)	3.288 (1)
30	50 (− 1)	5 (0)	20 (− 1)	340 (0)	2.740 (0)
31	65 (0)	6 (1)	25 (0)	340 (0)	3.288 (1)
32	80 (1)	5 (0)	25 (0)	320 (− 1)	2.740 (0)
33	65 (0)	6 (1)	30 (1)	340 (0)	2.740 (0)
34	50 (− 1)	5 (0)	30 (1)	340 (0)	2.740 (0)
35	80 (1)	5 (0)	25 (0)	340 (0)	3.288 (1)
36	65 (0)	6 (1)	25 (0)	360 (1)	2.740 (0)
37	65 (0)	5 (0)	30 (1)	320 (− 1)	2.740 (0)
38	80 (1)	5 (0)	25 (0)	340 (0)	2.192 (− 1)
39	50 (− 1)	5 (0)	25 (0)	360 (1)	2.740 (0)
40	65 (0)	5 (0)	20 (− 1)	360 (1)	2.740 (0)
41	65 (0)	6 (1)	25 (0)	320 (− 1)	2.740 (0)
42	65 (0)	4 (− 1)	30 (1)	340 (0)	2.740 (0)
43	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
44	65 (0)	5 (0)	25 (0)	340 (0)	2.740 (0)
45	65 (0)	5 (0)	20 (− 1)	340 (0)	3.288 (1)
46	65 (0)	4 (− 1)	20 (− 1)	340 (0)	2.740 (0)

Table 3 ANOVA and regression analysis for various responses

Parameters	MC (% d.b)	FGP (%)	SL (cm)	SDW (mg)	VI-I	VI-II	EC (mS/cm)
Intercept	73.58	72.11	30.49	182.35	2204.26	13,162.35	0.031
X_1	− 21.68***	13.47***	3.36***	9.66***	623.23***	3026.78***	0.004***
X_2	6.15***	4.63***	5.24***	15.03***	529.69***	1933.03***	0.006***
X_3	2.82**	− 0.67	0.59	1.70	25.59	12.97	0.003***
X_4	0.12	0.58	0.42	0.84	48.17	160.71	0.002**
X_5	− 1.07	0.45	0.56	0.90	49.43	122.46	0.002*
$X_1 X_2$	− 0.30	− 1.09	− 0.05	− 7.20**	13.97	− 601.65	0.000
$X_1 X_3$	0.74	0.25	− 0.09	− 0.51	− 5.27	− 3.87	0.002
$X_1 X_4$	0.61	− 0.83	0.25	0.14	2.53	− 117.76	− 0.003
$X_1 X_5$	− 1.21	0.16	0.30	0.85	45.47	142.79	0.001
$X_2 X_3$	− 1.98	0.25	1.02	3.15	77.80	270.57	0.002
$X_2 X_4$	− 0.35	0.59	0.91	0.79	84.21	161.27	0.002
$X_2 X_5$	1.28	− 0.49	− 0.57	− 2.91	− 63.64	− 311.05	0.000
$X_3 X_4$	− 2.52	− 0.42	0.05	− 0.13	− 7.78	− 76.97	0.000
$X_3 X_5$	0.54	− 0.25	− 0.04	1.55	− 8.62	74.31	0.000
$X_4 X_5$	− 2.48	− 0.17	0.57	2.20	36.93	131.16	0.000
X_1^2	− 17.46***	− 1.12*	− 1.21*	− 3.49*	− 78.44	− 332.75	0.003**
X_2^2	0.97	0.91	2.04***	6.40***	191.58***	684.20***	0.000
X_3^2	3.07	− 0.23	− 2.11***	− 8.87***	− 157.69***	− 686.35***	− 0.001
X_4^2	1.02	0.05	− 0.75	− 5.35***	− 50.15	− 368.86*	− 0.001
X_5^2	1.32	− 0.03	− 0.98	− 5.99***	− 72.91	− 433.23*	0.001
ANOVA							
R^2	0.94	0.97	0.91	0.91	0.95	0.95	0.79
Model *F* value	20.65***	45.40***	12.84***	13.58***	24.23***	28.56***	4.82***
Lack of fit (*p* value)	0.0088	0.2563 (NS)	0.3444 (NS)	0.0496	0.3945 (NS)	0.1338 (NS)	0.001
C.V (%)	7.62	2.64	5.88	2.88	7.26	4.88	11.82

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* vigour index-I, *VI-II* vigour index-II, *EC* electrical conductivity, *CV* coefficient of variation

*Significant at 10% ($p < 0.1$); **Significant at 5% ($p < 0.05$); ***Significant at 1% ($p < 0.01$)

coefficient of determination ($R^2 \geq 0.79$). On analysing the F values, it reflected that all the models (MC, FGP, SL, SDW, VI-I, VI-II and EC) were significant. Coefficient of variation (CV) values was less than 10% which established that the experiments were having reasonable accuracy and the models could be reproducible except for EC parameter. Lack of fit was found insignificant for FGP, SL, VI-I and VI-II parameters. The *p* values were used as a tool to check the significance of each of the coefficients, which in turn indicate the pattern of interactions between the variables. Smaller the '*p*' value of any particular coefficient, it is considered to be highly significant [16]. The insignificant terms from all second-order model equations were eliminated to get simplified predictive equation for all responses.

Moisture Content after Priming (MC)

Seed moisture has an important role in determining in vigour and viability of seeds. Evaluation of moisture content of the seeds after priming is a measure of its corresponding water potential. Moisture content was found in range of 32.45–86.72 (% d.b.) throughout the experimental run. Predicted response model shown in Table 3 indicated that linear effects of variables, viz. IGP and SD ($P \leq 0.01$) and temperature ($P \leq 0.05$) and quadratic effect of IGP ($P \leq 0.01$), were the primary determining factors. It can be revealed from regression analysis that IGP had negative linear effect on moisture content of seeds post-priming, i.e. moisture absorption capability of low vigourous seeds was observed more as compared to fresh seeds. Response surface plot (Fig. 1) also depicted that seeds with higher initial germination tend to absorb less moisture during hydropriming, while the increase in SD and temperature increases the moisture of exposed seeds. It might be due to the fact that seed lots exposed to longer duration of accelerated ageing tend to increase the permeability of seed's cellular membrane and thereby allowing for more moisture absorption while undergoing priming [19]. The inherent moisture content of the seed has substantial effect on its hardness [5], and hence, the seeds subjected to longer duration of hydropriming tend to become softer. Among the independent process variables, SD had highest effect, while IGP had least effect on MC as evident from regression coefficient (Table 3).

Data obtained from the three-level BBD matrix yielded following regression equation (Eq. 2), which was an empirical relationship between moisture content after hydropriming and the test variables in coded form:

$$\mathrm{MC} = 73.58 - 21.68X_1 + 6.15X_2 + 2.82X_3 - 17.46X_1^2 \quad (2)$$

Final Germination Percentage (FGP)

Estimation of FGP is considered to be of prime importance as it illustrates the success/failure of the hydropriming treatment given to aged seed lots. FGP increased with initial increase in the SD, which was followed by a decline due to prolonged soaking. Regression analysis (Table 3) showed that linear effect of IGP and SD had significant effect ($P \leq 0.01$) on FGP of seeds, while none of the interaction effect was found significant. However, the quadratic effect of IGP of seeds also found to be significant ($P \leq 0.01$). Response curves (Fig. 2) show a steep increase in germination with SD as compared to process temperature. This might be due to the fact that the driving force for water uptake during imbibition increases with soaking duration which in turn helped in increased size of cell walls with profound effect on transpiration and stomatal behaviour as also observed by May [21].

Quadratic model was selected for FGP response, and Eq. (1) was fitted in the experimental data. Proportional increase in FGP with initial germination was observed, but rate of germination was faster in low vigourous seeds (i.e. L_2 and L_3) when compared with seeds which are highly vigourous (L_1). This may be attributed to positive response of low vigour seeds to hydropriming rather than high vigour seeds. Further, hydropriming treatment might have brought about physico-chemical changes [12]. The fastest rate of germination was obtained by soaking seeds in water, probably due to faster water uptake and earlier initiation of metabolism processes, which determine radicle protrusion [10]. Significant terms in the form of equation can be written as follows (Eq. 3):

$$\mathrm{FGP} = 72.11 + 13.47X_1 + 4.63X_2 - 1.11X_1^2 \quad (3)$$

Seedling Length (SL)

SL values ranged from 23.16 to 38.83 cm for overall 46 experimental runs. It can be observed that linear effects of IGP and SD significantly affected ($P \leq 0.01$) seedling length. However, the quadratic effect of SD and temperature also found to be significant ($P \leq 0.01$) along with quadratic effect of IGP ($P \leq 0.1$). The interaction effect of none of the process variables found affecting the SL (Table 3).

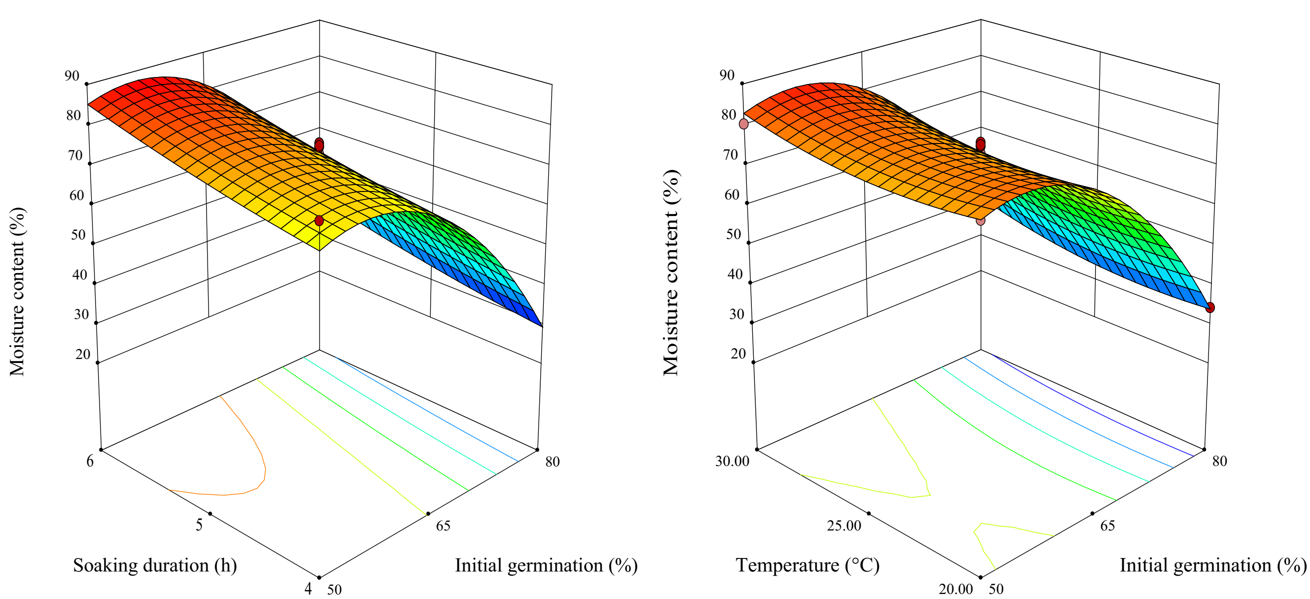

Fig. 1 Response surface plot of moisture content as a function of initial germination percentage, soaking duration and temperature

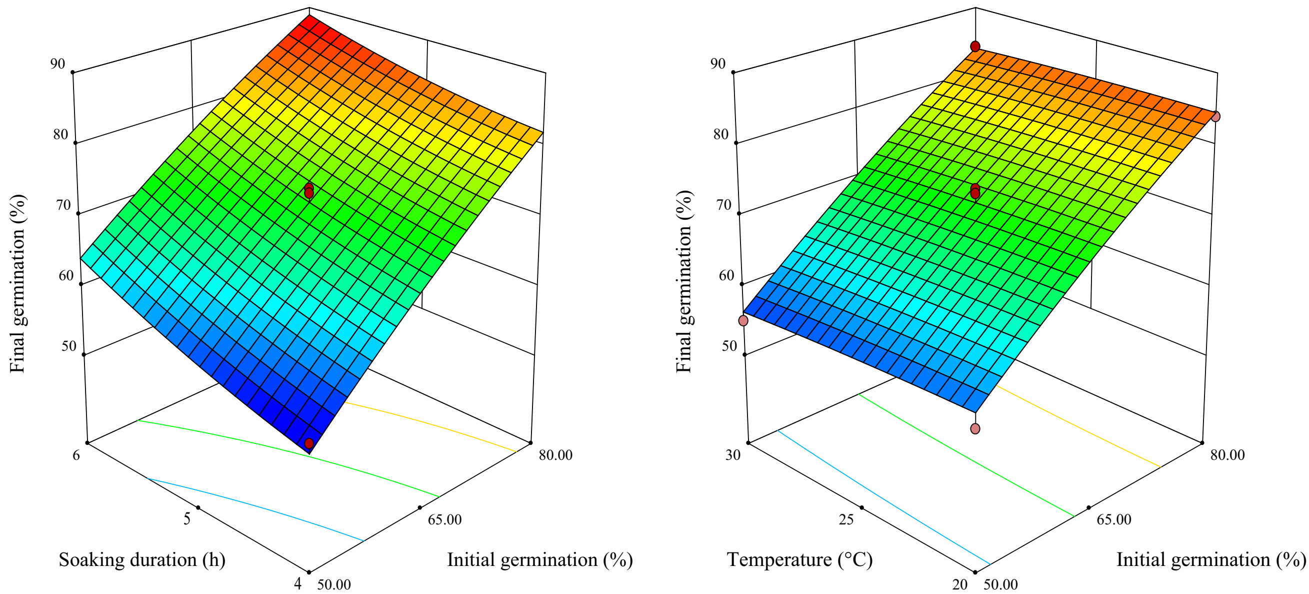

Fig. 2 Response surface plot of final germination of seeds as a function of initial germination percentage, soaking duration and temperature

As shown in surface plot (Fig. 3), SL increased steeply with SD as compared to the increase with IGP as expected. This increase might have resulted owing to absorption of more water due to increase in the elasticity of cell wall and development of a stronger and efficient shoot and root system [27]. The overall second-order polynomial equation with significant terms can be written as follows (Eq. 4):

$$\mathrm{SL} = 30.49 + 3.36X_1 + 5.24X_2 - 1.21X_1^2 + 2.04X_2^2 - 2.11X_3^2 \quad (4)$$

Seedling Dry Weight (SDW)

The values of SDW ranged from 158.65 to 204.06 mg/10 seedlings. The regression analysis results (Table 3) substantiated that IGP and SD had direct linear effect ($P \leq 0.01$), while their interaction showed a bit lower effect ($P \leq 0.05$). The quadratic effect of SD, temperature, rpm and AFR was reasonably higher ($P \leq 0.01$) as compared to seeds initial germination effect ($P \leq 0.1$). With respect of both linear and quadratic effects, SD had a more prominent effect than other variables and was justified from regression coefficient values.

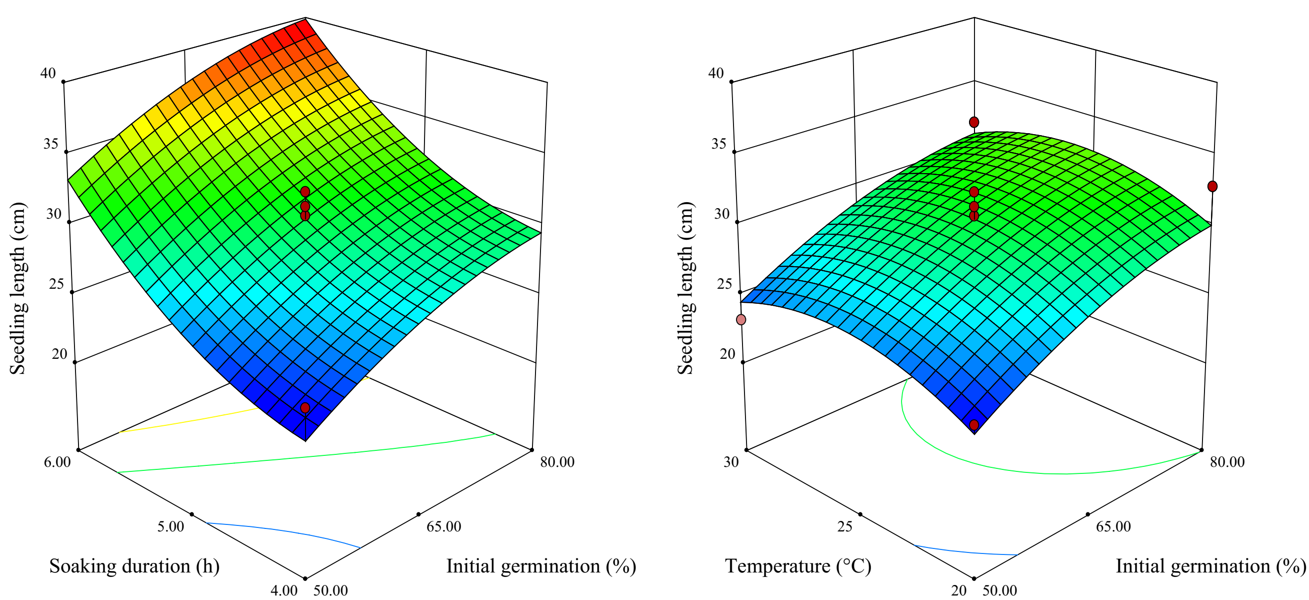

Fig. 3 Response surface plot of seedling length as a function of initial germination percentage, soaking duration and temperature

Curved surface on the surface plot (Fig. 4) reflected the significant effect of SD and AFR with proportional increase both the parameters. This trend was attributed towards increase in germination with priming duration and thereby resulting in healthier seedlings as compared to those less exposed to priming conditions. Good air flow rate was also a significant factor in increasing seed quality. Similar findings were also reported in lentil [10] and cowpea [6]. However, SDW reduced at higher values of temperature, because optimum temperature for better seed performance of okra seeds supposed to be around 30 ± 5 °C [17]. The model obtained for SDW was as follows (Eq. 5:

$$SDW = 182.36 + 9.65X_1 + 15.03X_2 - 7.20X_1X_2 - 3.49X_1^2 + 6.40X_2^2 - 8.87X_3^2 - 5.35X_4^2 - 5.99X_5^2 \quad (5)$$

Vigour Index-I (VI-I)

Primed seed lots of okra exhibited VI-I from 1273.8 to 3106.4. The regression analysis results (Table 3) showed IGP and SD had significant linear effect ($P \leq 0.01$), also quadratic effect of SD and temperature found to be highly considerable ($P \leq 0.01$). There was no significant interaction effect among any variables. Response surface plot (Fig. 5) shows an obvious increase in VI-I with increase in IGP and SD. This increase was because due to priming effect of low vigour seeds with long-lasting time period. Result of regression analysis in the form of equation can be written as (Eq. 6):

$$\text{VI - I} = 2204.16 + 623.23X_1 + 529.69X_2 + 191.58X_2^2 - 157.69X_3^2 \quad (6)$$

Vigour Index-II (VI-II)

Predicted response model (Table 3) indicated that linear effects of IGP and SD and quadratic effects of SD, temperature, rpm and AFR were the primary determining factors. None of interaction effect was found significant. VI-II was increasing with SD and IGP from lower to higher values of 8697.78–16,409.9. Response surface plot (Fig. 6) shows proportional increase in VI-II with SD, IGP and temperature which is an associated effect of increase in germination percentage of seed lots post-hydropriming. Second-order polynomial equation for VI-II can be written as follows (Eq. 7):

$$VI{-}II = 13162.35 + 3026.78X_1 + 1933.03X_2 + 684.20X_2^2 - 686.34X_3^2 - 368.86X_4^2 - 433.23X_5^2 \quad (7)$$

Electrical Conductivity (EC)

EC of okra seeds ranged from 0.022 to 0.0475 mS/cm. The linear terms of all five independent variables were significant at different levels (Table 3). Among all, IGP, SD and temperature were highly significant ($P \leq 0.01$), rpm ($P \leq 0.05$) a bit lower and AFR least significant ($P \leq 0.1$). While IGP was the only parameter affecting in quadratic terms ($P \leq 0.05$).

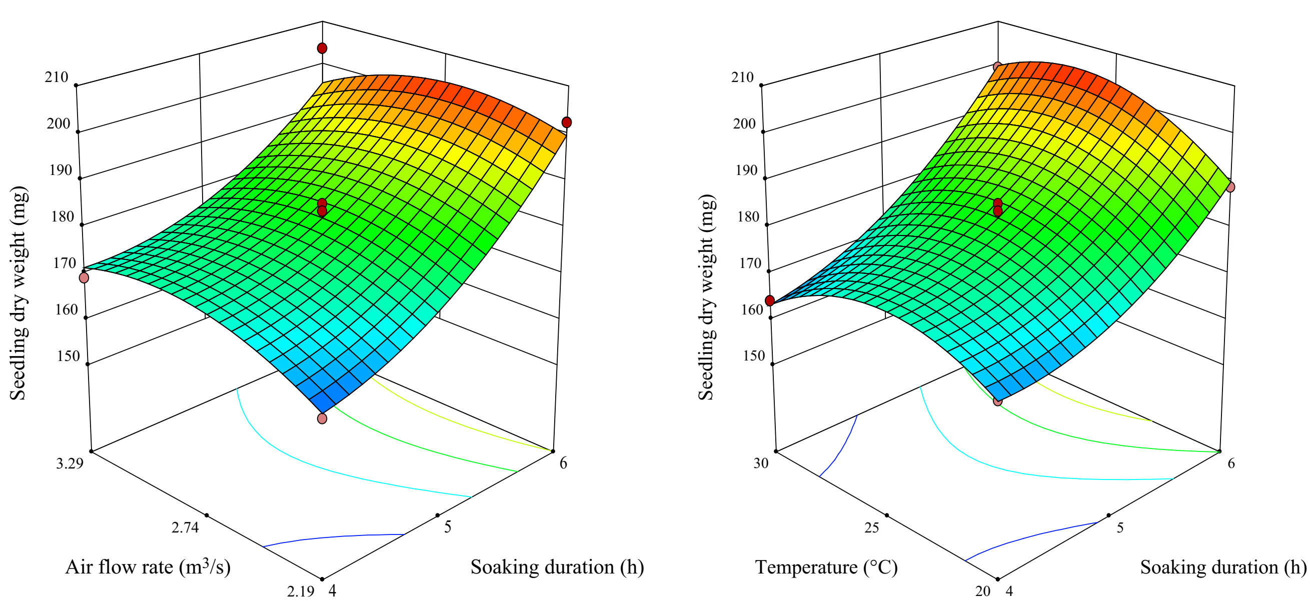

Fig. 4 3-D response surface plots showing effect of soaking duration, temperature and air flow rate on seedling dry weight

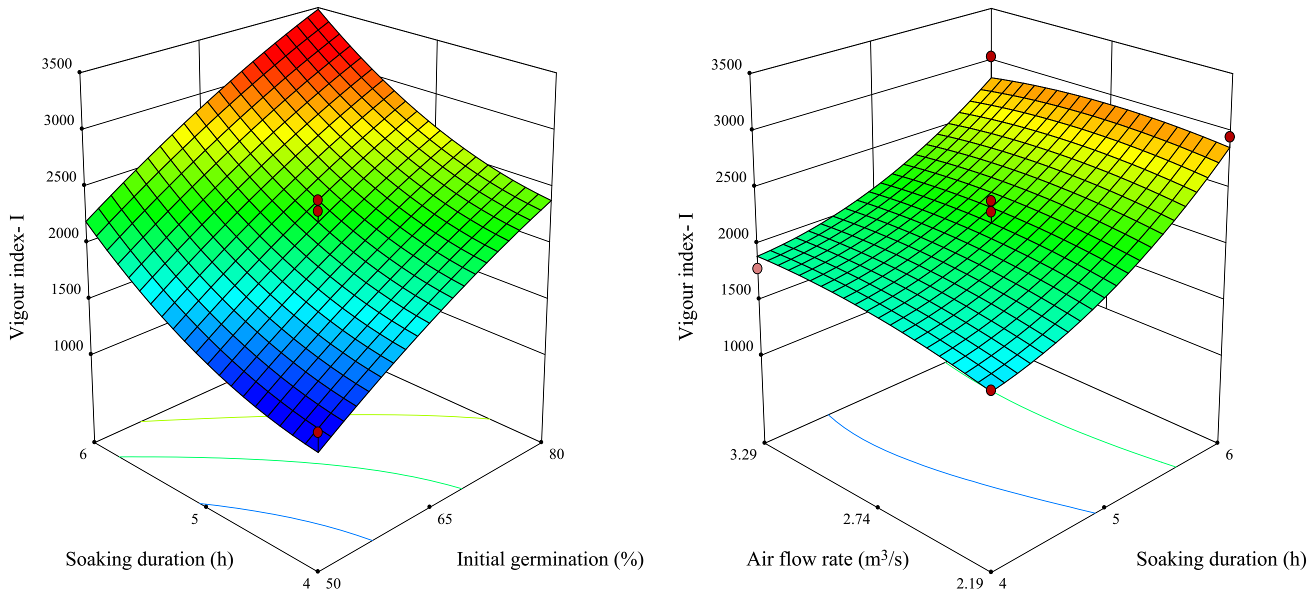

Fig. 5 3-D response surface plots showing effect of initial germination, soaking duration and air flow rate on VI-I

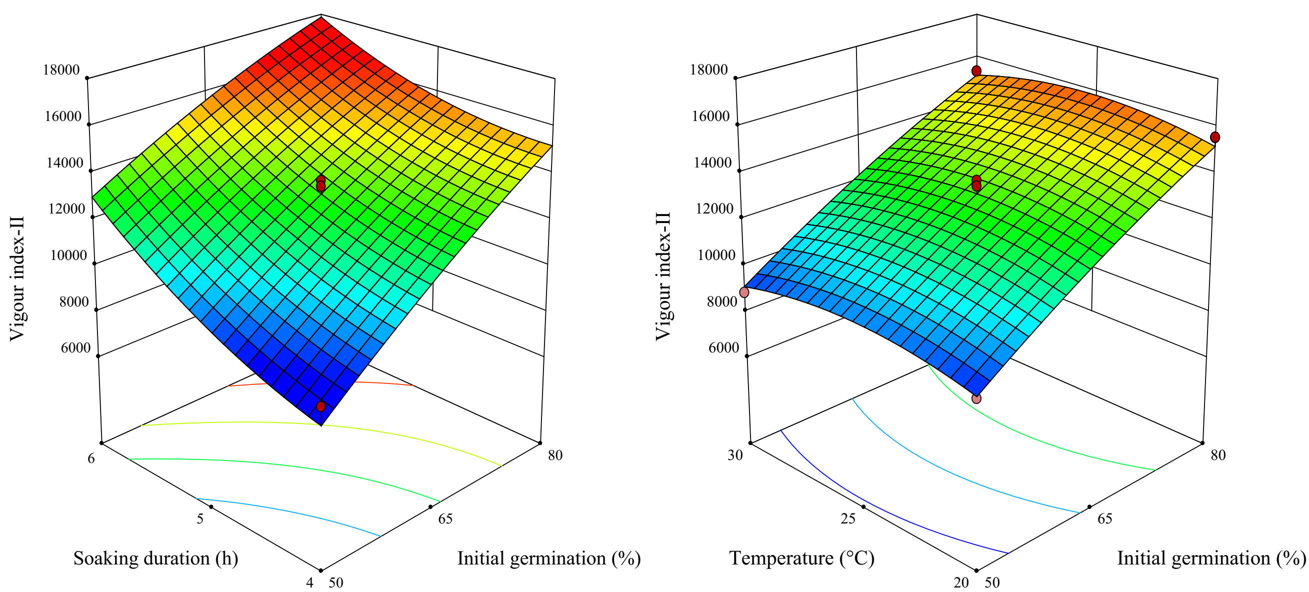

Fig. 6 3-D response surface plots showing effect of initial germination, soaking duration and temperature on VI-II

Response surface plot (Fig. 7) shows reduction in EC with increase in germination of seeds, also EC values found increasing with SD, temperature and rpm. The variation noticed in both high and low vigour seeds may be due to anatomical structure, permeability of membrane and difference in seed coat composition low vigour seeds leached more electrolytes which might be due to cellular membrane deterioration. Similar variation was noticed by Yogalakshmi et al. [30] in rice. Result of regression analysis was as follows (Eq. 8):

$$\begin{aligned} \mathrm{EC} = 0.031 &+ 3.843\,E - 003X_1 + 5.867\,E - 003X_2 \\ &+ 3.248\,E - 003X_3 + 2.019\,E - 003X_4 + 1.547\,E \\ &- 003X_5 - 17.46X_1^2 \end{aligned} \tag{8}$$

Radiography

Samples after hydropriming for all 46 experiments were subjected to X-ray imaging. Images show that seeds having more exposure of soaking duration and rotational speed were highly damaged as compared to those exposed for

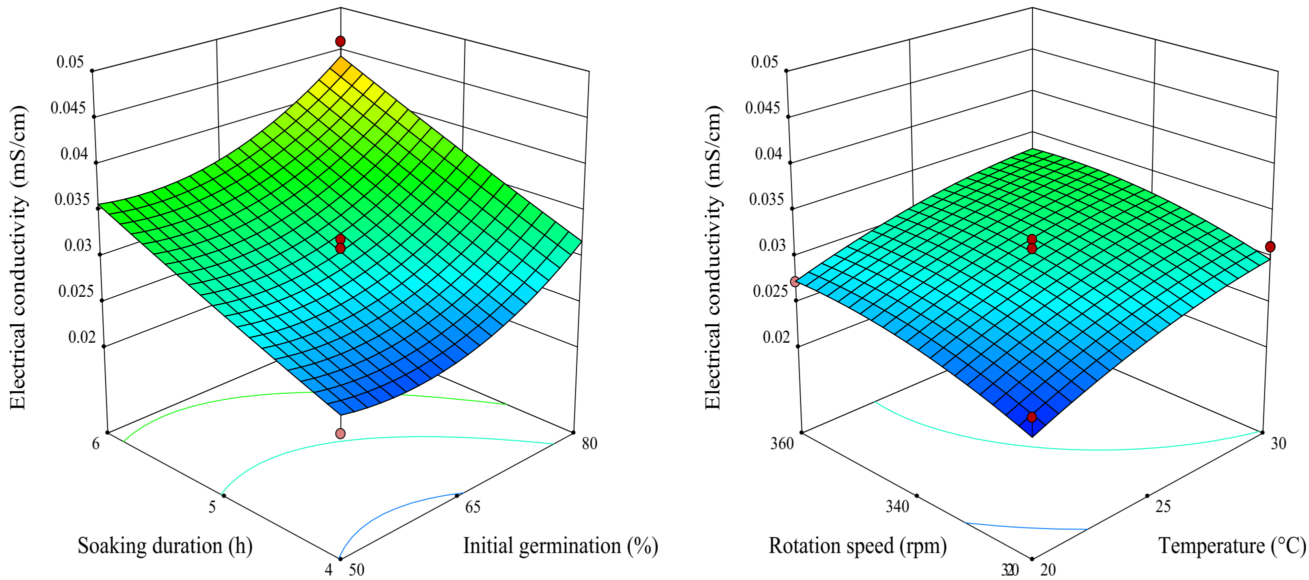

Fig. 7 3-D response surface plots showing effect of initial germination, soaking duration and rotation speed on electrical conductivity

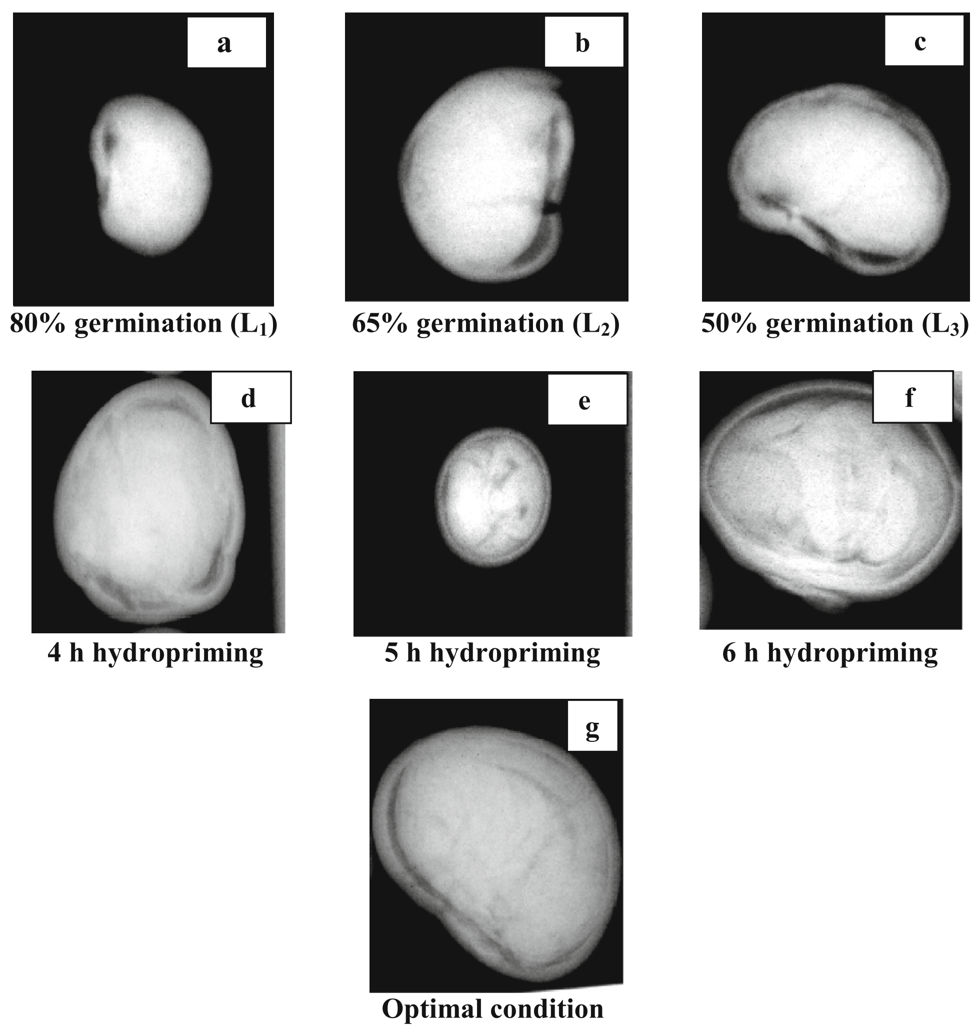

Fig. 8 Radiography view of okra seeds classified as: **a** L_1 (80% germination) **b** L_2 (65% germination) **c** L_3 (50% germination) **d** seed after 4 hour of hydropriming with minimal air cavity **e** seed after 5 hour of hydropriming with noticeable air cavity **f** seed after 6 hour of hydropriming showing prominent presence of visible cavities **g** hydroprimed seed at optimum conditions with nominal air cavity and structural variation in seed coat

Table 4 Comparison of experimental values with predicted values

Response	Predicted value	Actual value ± SD	SE ($n = 1$)	95% PI low	95% PI high
MC	73.58	72.58 ± 5.32	5.74	61.76	85.41
FGP	72.11	71.02 ± 1.90	2.05	67.88	76.33
SL	30.49	31.17 ± 1.73	1.86	26.65	34.33
SDW	182.36	184.05 ± 5.06	5.46	171.12	193.60
VI-I	2204.26	2213.69 ± 155.19	167.43	1859.34	2548.98
VI-II	13162.35	13,071.23 ± 620.87	669.80	11,782.88	14,541.83
EC	0.031	0.035 ± 0.004	0.004	0.022	0.039

lesser duration. This was due to seed coat disruption and also higher water absorption while priming. The changes in the internal seed structure after hydropriming are depicted in Fig. 8.

Optimization and Validation of Solution

Optimum values for process variables were obtained using Design Expert 8.0.7.1 by setting goals for all independent as well as dependent parameters. Numerical optimization was done by keeping final moisture content in range, maximizing FGP, SL, SDW, VI-I & VI-II and minimizing EC. These constrains resulted in the optimum conditions for hydropriming of okra seeds using the developed prototype as, initial germination 73%, soaking duration 6 h, temperature 23 °C, rotation speed 330 rpm and air flow rate 2.50 m^3/min.

Experiment was carried out at optimized conditions to validate the optimal solution given by the software. The hydroprimed seeds were subjected to quality assessment in terms of various parameters. Values of all dependent parameters were found at par with predicted values (Table 4). Overlaying contour plot (Fig. 9) shows the combined values for each of the responses in controllable factors space. The shaded yellow portion is the area which makes the collectively best possible value for all responses.

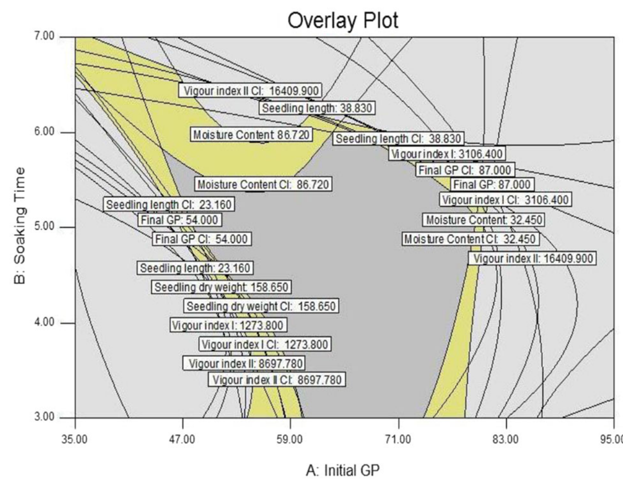

Fig. 9 Overlay plot of optimized conditions based on seed quality attributes

Conclusions

The developed seed priming prototype was tested for hydropriming of okra during this course of work. Designed experiments using BBD successfully exhibited the effect or process variables on the selected responses. Model analysis with the help of various relevant statistical aids, such as F value, coefficient of determination (R^2), CV and lack of fit, revealed that all the models were significant. Priming conditions, viz. IGP, SD, temperature, rpm and AFR, significantly affected seed quality parameters. Optimum solution for the overall experimental process was initial germination percentage 73, soaking duration 6 h, temperature 23 °C, rotation speed 330 rpm and air flow rate 2.50 m^3/min. Significant enhancement was observed in all seed quality attributes at optimum operating conditions. The developed priming equipment can be tested for different priming techniques with necessary modifications in the design.

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