



## **Effect of Germination on Vitamin C Content and Amylolytic Activity of Quinoa (*Chenopodium quinoa* Willd)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MNSS has performed the research analysis, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BAK has designed the study, guided in writing the protocol and first draft of the manuscript. Authors KUM and KBSD have monitored the proof reading of the article and author WJS has managed literature search. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To study the parameters for recording speed of germination, vitamin C and amylolytic changes in quinoa over a period of 72 h.

**Sample:** Whole (HGQ) quinoa grain (*Chenopodium quinoa* Willd) was procured from Department of Agronomy, College of Agriculture, PJTS Agricultural University, Rajendranagar, Hyderabad and commercially processed quinoa seed (CGQ) was purchased from local market was studied for the germination capacity after dehulling.

**Study Design:** Analyzing the germination speed parameters and vitamin C, amylolytic changes over 72 h of time period.

**Place and Duration of Study:** Analysis was conducted in PGRC Laboratory, PJTSAU, Rajendranagar, Hyderabad.

**Results:** Quinoa has strong germination capacity. The result indicates that higher FGP was seen at 20°C (83%) followed by 15°C (81%) and 25°C (80%). The radicals protruded after 4hrs of imbibition and reached to maximum length at 68hrs for 15°C and 25°C, where as it continued up to 72hrs for 20°C. Box plot diagram showed the median values at 20°C temperature ranged between 0.95 to 36.95 where as at 15°C it was 0.41 to 29.1 and 0.69 to 33.55 in 25°C indicating maximum viability of the grain at 20°C temperature. The mean scores of vitamin C content was high at 20°C temperature at all stages when compared to other two temperatures i.e. 15°C and 25°C. It showed that  $\alpha$ -amylase activity was developed in germinated samples and rapidly increased up to 32hrs of imbibition and slightly decreased after 48hrs of germination.

**Conclusion:** Quinoa has high germinated capacity as it started from 4hrs upon germination.

**Keywords:** Germination; functional properties; vitamin C; amylolytic activity; imbibition; box-plot diagram.

## 1. INTRODUCTION

Interestingly quinoa has short germination period of 4-5 hours in comparison to other grains which require at least 12-14 hours germination process overnight. Germination of quinoa enhances the nutrient content and reduces antinutritional factors like saponins [1].

Methods of evaluating seed germination responses may be categorized as analytical or graphical. Germination is traditionally considered to be a qualitative developmental response of an individual seed that occurs at a point in time [2].

Kaur and Tanwar [3] studied vitamin C content, total polyphenols and antioxidant activity in Indian *Chenopodium quinoa* seeds grown in Anantapur district. The results revealed that domestic processing of quinoa seeds mainly by the process of germination enriched its vitamin C, polyphenols content and antioxidant activity. It can be inferred from the present study that domestically processed quinoa by germination could be preferred over industrially processed quinoa.

Hager et al. [4] studied the development of amylolytic activities and subsequent changes in sugar profiles and starch content were followed in quinoa over a period of 72 h. A low level of  $\alpha$ -amylase activity (determined at  $P^H$  5.2) was present in the embryo of non-germinating seeds, but emerged in the perisperm only after 24 h. Study shows that the levels of amylolytic activities remained very low compared with traditional malting cereals, suggesting the unsuitability of quinoa as a source of amylases in food applications.

Research on quinoa has mainly focused on composition of the whole seed, protein quality, starch functionality and incorporation of quinoa into food products, the current study aimed to evaluate the influence of different time and temperature conditions on quinoa seed germination.

## 2. MATERIALS AND METHODOLOGY

### 2.1 Procurement of Raw Materials

Quinoa seeds were obtained from Department of Agronomy, College of Agriculture, PJTSAU Agricultural University, Rajendranagar, Hyderabad. The other ingredients were procured from local market of Hyderabad. The glassware and equipment were available at Post Graduate & Research Centre, PJTSAU, Rajendranagar, Hyderabad, were used throughout the study.

### 2.2 Standardization of Time and Temperature Protocols for Germination of Quinoa

For the germination tests, petriplates were cleaned and rinsed with distilled water and sterilized. Before initiating the tests, the seeds were immersed in 10 ml of hydrogen peroxide for 10 minutes and rinsed with distilled water to avoid fungal infection. Seeds were soaked in 15 ml of distilled water for 10 hrs, after completing the soaking time the seeds were shifted to sterilized petri plates which were covered with filter paper and were placed in biochemical oxygen demand (BOD) chambers at three constant temperatures of 15, 20, 25°C respectively. Three replications of 100 seeds for each temperature were evaluated.

The germinated seeds were counted from 4<sup>th</sup> hour after keeping in the BOD chamber. The experiment was finished when no further radical protrusions were observed after an average of 72 hours. For every 4 hrs intervals quinoa seeds were assessed for germination parameters, vitamin C content and alpha amylase activity.

### 2.3 Germination Parameters

The germinated (GRS), abnormally germinated (AGS) (in which the cotyledons emerge before the radical) and non-germinated seeds (NGS) were counted after 4 hrs and expressed as percentage from the total number of seeds [4]. The protrusion radical of 10 seeds from each petridish was measured at each time point using a digital caliper.

### 2.4 Parameters to Assess the Speed of Quinoa Seed Germination

In the present study the pattern of germination of quinoa seeds were assessed by Al-Mударis [2] method. Vitamin C content was determined by following the method of Harris and Ray [5] and the amylase activity was assayed using the method of Bernfeld [6] to measure reducing sugar groups released by the enzyme.

4, 6 and 8 and 12 hrs germinated quinoa was cooked by using pressure cooker (50 gm germinated quinoa in 100 ml water for 8min) was given for determining the sensory characteristics of germinated quinoa. A semi-trained panel of 15 members from PGRC, PJTSAU using 9 point hedonic scale evaluated the colour, texture, flavour, taste and overall acceptability of germinated and cooked quinoa samples on 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> hour of incubation at 15°C, 20°C, 25°C respectively. Scores were based on a hedonic scale of 1 to 9 where: 1=I dislike extremely (very bad) and 9 = I like extremely (excellent) [7].

### 2.5 Statistical Analysis

All the results were statistically analyzed to test the significance of the results using percentages, means, standard deviations and co-relation coefficient [8]. All the analysis was performed in replications and the results were presented as mean  $\pm$  standard deviation. Difference between the variables was tested for significance by (ANOVA) using SAS version 9.1.

## 3. RESULTS AND DISCUSSION

Sprouts of germinated quinoa were noticeable after 4hrs only. Atwell et al. [9] reported that sprouting was observed after 6hrs of germination where as Hager et al. [4] stated that sprouting was seen after 8hrs of germination. Difference between studies may be due to variety difference, physiological quality of seeds and environmental conditions and germination requirements like water, temperature and light [10].

### 3.1 Germination Capacity

The mean scores of percentage of germinated seeds at 15°C ranged from 28.33 $\pm$ 4.70 to 81.00  $\pm$ 1.53%, non-germinated seeds ranged from 70.00 $\pm$ 3.61 to 17.67 $\pm$ 1.33% and abnormally germinated seeds 0.33 $\pm$ 0.33 to 1.33 $\pm$ 0.33%. The germination of seeds at 15°C was maximum during 24hrs (81%) and from then there was no change in number of seeds germinated (Table 1).

The mean scores of percentage of germinated seeds at 20°C ranged from 30.33 $\pm$ 7.45 to 83.33  $\pm$ 0.67%, non-germinated seeds ranged from 69.67 $\pm$ 7.45 to 15.33 $\pm$ 0.33% and abnormally germinated seeds 0.00 $\pm$ 0.00 to 1.33 $\pm$ 0.33%. The germination of seeds at 20°C continued till 72hrs (83.33%) (Table 1).

The mean scores of percentage of germinated seeds at 25°C ranged from 22.67 $\pm$ 11.39 to 80.00  $\pm$ 1.00%, non-germinated seeds ranged from 77.33 $\pm$ 11.39 to 10.00 $\pm$ 2.89% and abnormally germinated seeds 0.00 $\pm$ 0.00 to 10.00 $\pm$ 3.05%. The number of seeds germinated was constant from 60hrs at 25°C (Table 1).

The maximum number of seeds germinated was statistically high ( $p \leq 0.05$ ) at 20°C, abnormally germinated seeds were low at 20°C indicating highest germination capacity at 20°C. Germination was stagnant after 24hrs at 15°C, 60hrs at 25°C where as it continued up to 72hrs at 20°C indicating suitability of this temperature for highest germination capacity. Only 1-2% of seeds showed abnormal germination at 15°C and 20°C where as it was up to 10% in 25°C (Fig. 1).

### 3.2 Germination Speed Parameters

The final germination percentage alone is unsatisfactory for reporting the results [2].

**Table 1. Mean scores of physical characteristics during germination of quinoa seeds**

Time (hrs)	15°C			20°C			25°C		
	GRS	NGS	AGS	GRS	NGS	AGS	GRS	NGS	AGS
4	28.33±4.70	70.00±3.61	0.33±0.33	30.33±7.45	69.67±7.45	0.00±0.00	22.67±11.39	77.33±11.39	0.00±0.00
6	29.67±3.93	70.00±3.61	0.33±0.33	48.00±8.50	52.00±8.50	0.00±0.00	34.67±11.85	64.67±12.17	0.66±0.33
8	29.67±3.93	51.33±15.68	0.33±0.33	48.00±8.50	52.00±8.50	0.00±0.00	41.33±10.91	58.00±11.24	0.66±0.33
12	64.67±6.49	35.00±6.81	0.33±0.33	66.67±2.33	33.00±2.65	0.33±0.33	71.67±2.33	27.67±2.03	0.66±0.33
16	74.67±2.33	25.00±2.65	0.33±0.33	74.00±1.73	24.67±1.76	1.33±0.33	72.67±2.19	26.33±1.67	0.66±0.33
20	77.67±0.67	22.00±1.00	0.33±0.33	78.00±1.00	20.67±1.33	1.33±0.33	73.00±2.08	26.00±1.53	0.66±0.33
24	81.00±1.53	17.67±1.33	1.33±0.33	78.33±0.88	20.67±1.33	0.66±0.66	75.00±2.08	26.00±1.53	0.66±0.33
28	81.00±1.53	17.67±1.33	1.33±0.33	78.33±0.88	20.67±1.33	0.66±0.66	75.67±2.08	23.67±2.40	1.33±0.33
32	81.00±1.53	17.67±1.33	1.33±0.33	79.33±1.33	19.00±1.53	0.66±0.66	77.00±1.67	23.00±2.00	1.33±0.33
36	81.00±1.53	17.67±1.33	1.33±0.33	80.67±1.20	19.00±1.53	0.33±0.33	77.00±1.53	16.67±2.40	6.33±0.88
40	81.00±2.08	17.67±1.33	1.33±0.88	80.67±1.20	18.00±1.15	1.33±0.33	77.00±1.53	16.67±2.40	6.33±0.88
44	81.00±2.08	17.67±1.33	1.33±0.88	81.33±1.45	17.33±1.45	1.33±0.33	77.00±1.53	16.67±2.40	6.33±0.88
48	81.00±2.08	17.67±1.33	1.33±0.88	81.33±1.45	17.33±1.45	1.33±0.33	77.00±5.53	13.67±2.60	9.33±3.33
52	81.00±1.53	17.67±1.33	1.33±0.33	82.00±1.15	16.67±1.20	1.33±0.33	79.00±1.53	11.67±2.60	9.33±3.33
56	81.00±1.53	17.67±1.33	1.33±0.33	82.33±0.88	16.33±0.88	1.33±0.33	79.67±1.20	11.00±2.60	9.33±3.33
60	81.00±1.53	17.67±1.33	1.33±0.33	82.67±0.67	16.00±0.58	1.33±0.33	80.00±1.00	10.00±2.89	10.00±3.05
64	81.00±1.53	17.67±1.33	1.33±0.33	82.67±0.67	16.00±0.58	1.33±0.33	80.00±1.00	10.00±2.89	10.00±3.05
68	81.00±1.53	17.67±1.33	1.33±0.33	83.00±0.58	15.67±0.33	1.33±0.33	80.00±1.00	10.00±2.89	10.00±3.05
72	81.00±1.53	17.67±1.33	1.33±0.33	83.33±0.67	15.33±0.33	1.33±0.33	80.00±1.00	10.00±2.89	10.00±3.05

Note: Values are expressed as mean ± standard deviation for all the determinants.

GRS – Germinated seeds

NGS – Non germinated seeds, AGS – Abnormally germinated seeds

**Table 2. Mean scores of germination speed parameter in germinated quinoa at three temperatures**

(a) 15°C

S.No	Parameter	FGP	MGT	FDG	LDG	TSG	GRI
1.	Score	70.60±0.50	0.03±0.01	5.00±0.57	25.00±0.58	21.67±0.88	147.8±0.40
2.	Mean	70.60	0.02	2.50	12.50	10.83	73.91
3.	CD	0.50	0.01	2.48	2.48	3.79	0.61
4.	SE of mean	2.47	0.01	1.14	5.59	4.86	33.05
5.	CV (%)	0.43	15.18	28.28	5.67	9.97	0.62

## (b) 20°C

S.No	Parameter	FGP	MGT	FDG	LDG	TSG	GRI
1.	Score	73.55±1.27	0.28±1.97	4.33±0.33	71.67±0.33	68.00±0.58	145.60±0.30
2.	Mean	73.55	-0.28	2.16	35.83	34.00	72.80
3.	CD	1.27	1.97	1.43	1.43	2.48	0.38
4.	SE of mean	1.93	0.22	0.98	16.02	15.20	32.55
5.	CV (%)	1.04	-419.1	18.84	1.13	2.08	0.54

## (c) 25°C

S.No	Parameter	FGP	MGT	FDG	LDG	TSG	GRI
1.	Score	69.79±0.00	0.02±0.00	5.66±0.88	61.67±0.88	55.00±0.58	137.50±0.30
2.	Mean	69.79	0.02	2.83	30.83	27.50	68.76
3.	CD	0.00	0.00	3.79	3.79	2.48	0.36
4.	SE of mean	2.20	0.01	1.32	13.79	12.30	30.75
5.	CV (%)	0.00	0.00	38.12	3.50	2.57	0.56

Note: Values are expressed as mean ± standard deviation for all the determinants; FGP – Final germination percentage; MGT – Mean germination time; FDG – First day of germination; LDG – last day of germination; TSG – Time spread of germination; GRI- Germination rate index

Table 3. Mean scores of length, vitamin C and alpha amylase content of germinated seeds

Time(hrs)	15°C			20°C			25°C		
	LS (mm)	VC (mg)	AA (µm)	LS (mm)	VC (mg)	AA (µm)	LS (mm)	VC (mg)	AA (µm)
4	0.33±0.08	4.21±0.12	0.15±0.02	1.00±0.05	0.91±0.09	0.51±0.02	0.66±0.02	7.20±0.05	0.40±0.00
6	1.03±0.26	5.43±0.07	0.25±0.02	2.10±0.15	14.07±0.32	0.56±0.02	1.84±0.03	16.23±0.17	0.50±0.00
8	2.06±0.34	6.47±0.13	0.35±0.03	3.06±0.20	20.95±0.22	0.61±0.01	2.40±0.05	21.52±0.31	0.57±0.00
12	3.40±0.30	7.89±0.15	0.44±0.02	5.23±0.20	24.06±0.31	0.67±0.02	4.43±0.12	24.63±0.31	0.62±0.00
16	4.80±0.49	10.22±0.25	0.52±0.01	7.30±0.26	25.37±0.39	0.71±0.04	6.19±0.12	25.32±0.45	0.70±0.00
20	7.50±0.32	14.24±0.26	0.59±0.02	9.26±0.18	28.97±0.25	0.78±0.01	8.29±0.21	28.74±0.25	0.74±0.00
24	9.43±0.50	17.38±0.24	0.63±0.02	11.13±0.33	32.22±0.38	0.84±0.01	10.24±0.32	31.14±0.25	0.79±0.00
28	11.16±0.44	19.04±0.29	0.67±0.02	13.12±0.28	33.43±0.28	0.92±0.01	12.05±0.43	31.96±0.37	0.82±0.00
32	13.80±0.97	25.86±0.45	0.72±0.02	16.30±0.30	35.18±0.18	0.95±0.02	16.29±0.25	33.13±0.23	0.89±0.00
36	15.38±1.17	26.48±0.23	0.78±0.02	18.33±0.33	35.99±0.36	1.06±0.01	17.63±0.31	34.93±0.17	0.90±0.00
40	18.76±1.67	28.23±0.37	0.83±0.02	22.80±0.43	43.94±0.21	1.12±0.02	22.10±0.11	35.84±0.04	0.98±0.00
44	20.86±1.65	29.89±0.29	0.85±0.02	23.56±0.29	54.98±0.37	1.18±0.01	23.50±0.28	35.98±0.33	1.02±0.00
48	25.33±1.46	31.70±0.25	0.79±0.02	28.63±0.31	69.62±0.32	1.29±0.01	27.83±0.44	43.55±0.49	1.04±0.00
52	26.96±1.46	31.93±0.23	0.71±0.01	31.33±0.33	77.42±0.11	1.41±0.02	29.63±0.31	48.72±0.29	0.95±0.00
56	28.56±0.77	32.23±0.28	0.66±0.01	32.00±0.57	77.70±0.25	1.48±0.02	29.56±0.29	51.00±0.56	0.82±0.00
60	27.86±1.03	32.23±0.33	0.60±0.01	35.00±0.57	78.26±0.53	0.91±0.02	30.66±0.44	57.79±0.56	0.74±0.00

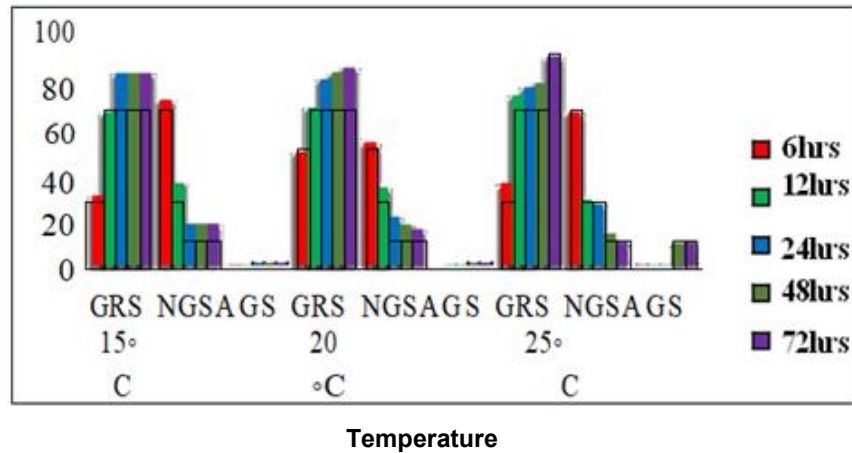
Time(hrs)	15°C			20°C			25°C		
	LS (mm)	VC (mg)	AA (µm)	LS (mm)	VC (mg)	AA (µm)	LS (mm)	VC (mg)	AA (µm)
64	28.33±0.88	32.27±0.32	0.51±0.02	37.00±0.57	81.13±0.18	0.80±0.03	30.66±0.44	57.79±0.56	0.61±0.00
68	29.44±0.55	32.29±0.34	0.48±0.01	37.00±0.57	81.60±0.35	0.74±0.02	31.96±0.29	57.79±0.56	0.54±0.00
72	29.40±0.55	32.29±0.34	0.39±0.01	37.00±0.57	81.80±0.49	0.66±0.01	33.16±0.44	57.7±0.56	0.42±0.00

Note: Values are expressed as mean ± standard deviation for all the determinants  
 LS: Length of sprouts in milli meter, VC: vitamin C in milligrams, AA: Alpha amylase in micro molar

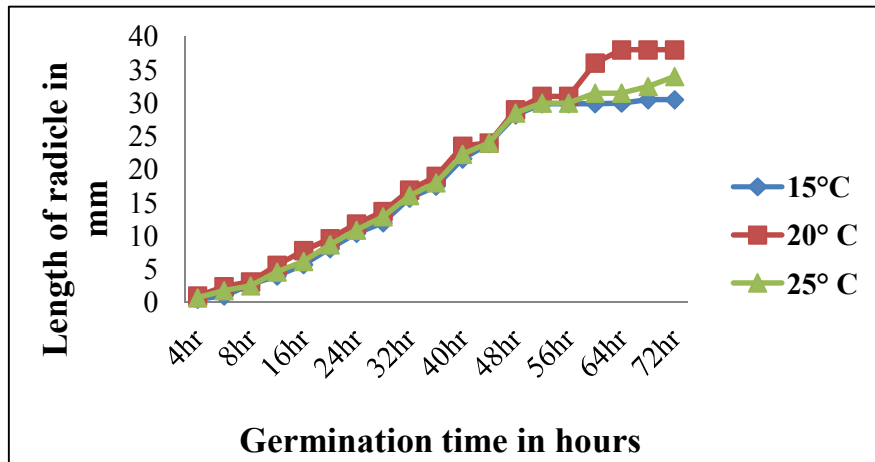
**Table 4. Pearson Correlation Coefficient between physical quality variables and germination parameters of germinated quinoa**

	LS -15°C	VC-15°C	AA-15°C	FGP-15°C	MGT-15°C	VC-20°C	AA-20°C	FGP-20°C	MGT-20°C	LS -25°C	VC-25°C	AA-25°C	FGP-25°C	MGT-25°C	LS-20°C
LS -15°C	1.00000	0.97723 ***	0.44182	0.70762 ***	-0.50227 *	0.97723 ***	0.44182	0.70762 ***	-0.50227 *	0.99786 ***	0.97723 ***	0.44182	0.70762 ***	-0.50227 *	0.98617 ***
VC-15°C		1.00000	0.59189 **	0.78886 ***	-0.54239 *	1.00000 ***	0.59189 **	0.78886 ***	-0.54239 *	0.97119 ***	1.00000 ***	0.59189 **	0.78886 ***	-0.54239 *	0.94893 ***
AA-15°C			1.00000	0.74794 ***	-0.26679	0.59189 **	1.00000 ***	0.74794 ***	-0.26679	0.40574	0.59189 **	1.00000 ***	0.74794 ***	-0.26679	0.33455
FGP-15°C				1.00000	-0.18339	0.78886 ***	0.74794 ***	1.00000 ***	-0.18339	0.70448 ***	0.78886 ***	0.74794 ***	1.00000 ***	-0.18339	0.68472 **
MGT-15°C					1.00000	-0.54239 *	-0.26679	-0.18339	1.00000 ***	-0.49512 *	-0.54239 *	-0.26679	-0.18339	1.00000 ***	-0.47426 *
VC-20°C						1.00000	0.59189 **	0.78886 ***	-0.54239 *	0.97119 ***	1.00000 ***	0.59189 **	0.78886 ***	-0.54239 *	0.94893 ***
AA-20°C							1.00000	0.74794 ***	-0.26679	0.40574	0.59189 **	1.00000 ***	0.74794 ***	-0.26679	0.33455
FGP-20°C								1.00000	-0.18339	0.70448 ***	0.78886 ***	0.74794 ***	1.00000 ***	-0.18339	0.68472 **
MGT-20°C									1.00000	-0.49512 *	-0.54239 *	-0.26679	-0.18339	1.00000 ***	-0.47426 *
LS -25°C										1.00000	0.97119 ***	0.40574	0.70448 ***	-0.49512 *	0.99329 ***
VC-25°C											1.00000	0.59189 **	0.78886 ***	-0.54239 *	0.94893 ***
AA-25°C												1.00000	0.74794 ***	-0.26679	0.33455
FGP-25°C													1.00000	-0.18339	0.68472 **
MGT-25°C														1.00000	-0.47426 *
LS-20°C															1.00000

Note: Significance Levels  $p < 0.05^*$   $0.01^{**}$   $0.001^{***}$   
 If correlation  $r =>$  0.45551 0.57504 0.69312



**Fig. 1. Percentage of germinated seeds at three different temperatures**  
 GRS – Germinated seeds; NGS – Non germinated seeds; AGS – Abnormally germinated seeds



**Fig. 2. Mean scores of sprout length of germinated quinoa seeds at three different temperatures**  
 Box and whisker plots for seed germination at 15°C, 20°C and 25°C temperature at different time periods

Higher FGP was seen at 20°C (83%) followed by 15°C (81%) and 25°C (80%) as shown in Table 2. The mean germination time (MGT) represents the mean time a seed lot requires to initiate and end germination. The lower the MGT, the faster a population of seeds has germinated.

The day on which the first germination event occurred (FDG) and the day on which the last germination event occurred (LDG) are also useful in germination speed studies. In the present study FDG occurs within hours so, the results are presented in hours instead of days. FDG occurs at 4.33±0.33 hours at 20°C where as it was 5.00±0.57 and 5.66±0.00 at 15°C and 25°C respectively (Table 2). The LDG was extended up to 71.67±0.33 for 20°C but it was

25.00 ±0.58 hrs for 15°C and 61.67 ±0.88 hrs for 25°C (Table 2).

The time in days (hrs) between the first and last germination events occurring in a seed lot is called as time spread of germination (TSG). The higher the TSG value, the greater the difference in germination speed between the fast and slow germinating seeds. In the present study the TSG was high at 20°C temperature (68.00±0.58hr) followed by 25°C (55.00±0.58) and lowest at 15°C (21.07±0.88) as shown in Table 2.

The germination rate index (GRI) basically gives an indication of the percentage of seeds germinating per day of the test run period. In the present study the GRI was high at 15°C

(147.8±0.4) followed by 20°C (145.6±0.30) and lowest at 25°C (137.5±0.3) as shown in Table 2.

### 3.3 Length of Radical, Vitamin C Content and Amylase Activity

#### 3.3.1 Length of the radicle

Quinoa seeds exhibited rapid growth when exposed to moisture and temperature. The radical protrusion was seen after 4hrs of imbibition.

The mean scores of length of radicals at 15°C ranged from 0.33±0.08 to 29.40±0.55 mm, at 20°C it was 1.00±0.05 to 37.00±0.57 and at 25°C it was 0.66±0.02 to 33.16±0.44 mm (Table 3). The mean scores at different time intervals at three temperatures (15°C, 20°C and 25°C) were taken and line graph was drawn.

The mean scores of length of radical was high at 20°C temperature at all stages when compared to other two temperatures i.e. 15°C and 25°C (Fig. 2). The radical length at 12hrs was 3.40±0.30 (15°C), 5.23±0.20 (20°C) and 4.43±0.12 (25°C) and at 72hrs it was 29.40±0.55 (15°C), 37.00±0.57 (20°C) and 33.16±0.44 (25°C). The mean scores were very high when compared to the study of Makinen [11], where they reported an average length of 1.7±0.4 mm after 24hrs and 35.0±6.3 mm after 72hrs. The radicals protruded after 4hrs of incubation and reached to maximum length at 68hrs for 15°C and 25°C, where as it continued up to 72hrs for 20°C (Fig. 2). The results were significantly different from the study of Hager et al. [4]. They have reported radical protrusion after 5hrs at

25°C and 9hrs at 5°C, showing strong temperature dependence.

Box plot diagram was drawn by taking the ten measurements for time point at each temperature to observe the radical elongation behavior during germination of quinoa. In box plot the median of the data (horizontal lines in the boxes) show that half the scores are greater than or equal to this value and half are less. The medium values at 20°C temperature ranged between 0.95 to 36.95 where as at 15°C it was 0.41 to 29.1 and 0.69 to 33.55 in 25°C indicating maximum viability of the grain at 20°C temperature.

The “middle box” represents the quartile ranges. 75% of scores fall below the upper quartile and 25% of scores fall below the lower quartile.

The 25<sup>th</sup> percentile of radicle elongation of ten seeds at different points of time at 15°C ranged from 0.31 to 28.10 and 75<sup>th</sup> percentile of radicle elongation ranged from 0.48 to 29.35 (Fig. 3a). The 25<sup>th</sup> percentile of radicle elongation of ten seeds at different points of time at 20°C ranged from 0.82 to 36.42 and 75<sup>th</sup> percentile of radicle elongation ranged from 1.17 to 37.5 (Fig. 3b). The 25<sup>th</sup> percentile of radicle elongation of ten seeds at different points of time at 25°C was ranged from 0.65 to 32.92 and 75<sup>th</sup> percentile of radicle elongation ranged from 0.73 to 33.95 (Fig. 3c). The short boxes in all the temperatures suggest that difference between overall radical lengths have concurrence with each other. The upper and lower whiskers show the range between minimum and maximum values.

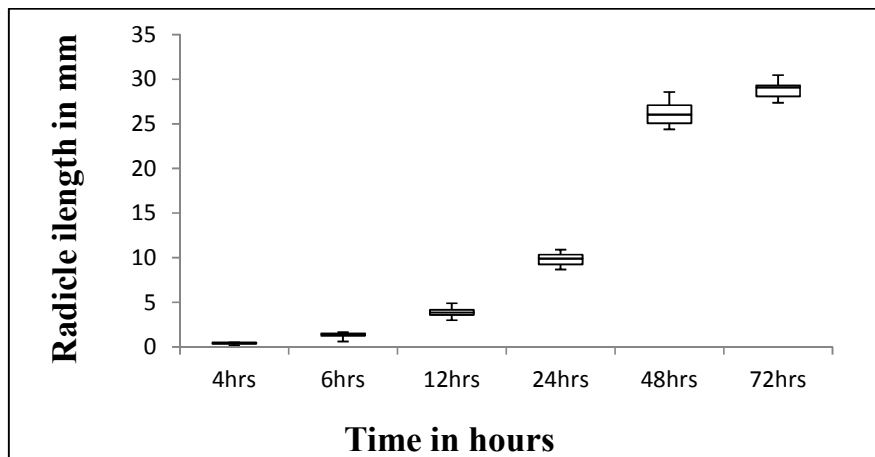


Fig. 3(a).



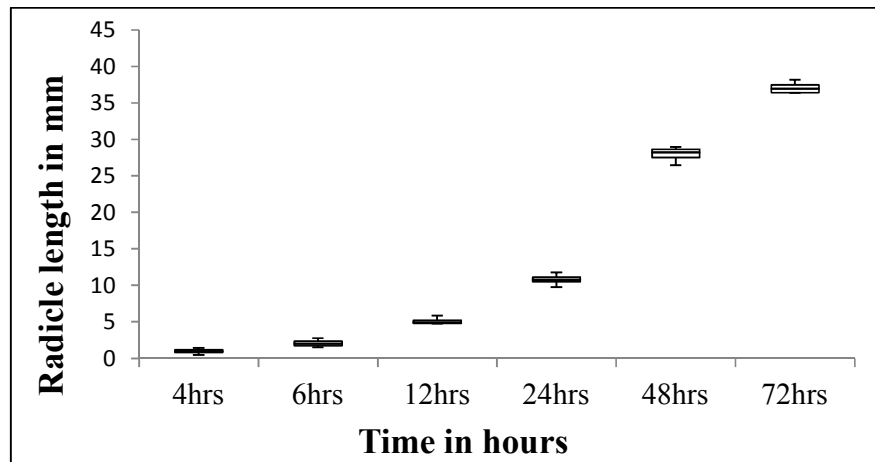


Fig. 3(b).

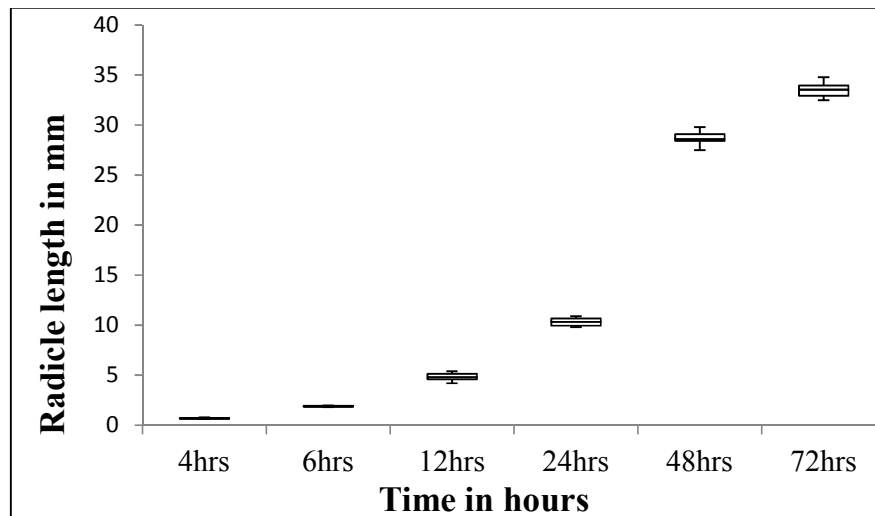


Fig. 3(c).

**Fig. 3. Box and whisker plots displaying median, inter quartile range (box) and range (whiskers) of percent seed germination of quinoa at three temperatures a) 15°C b) 20°C c) 25°C**

Box and whisker plots show that quinoa had the highest germination percentage at 20°C (Fig. 3b). The median and inter quartile ranges differed among the temperatures and different time periods. However, in all the temperatures it was observed that 75% of the inter quartile range is towards higher radicle length at 48hrs of germination (Fig. 3c).

### 3.3.2 Vitamin C content of the radicle

The mean scores of vitamin C content of radicles at 15°C ranged from  $4.21 \pm 0.12$  to  $32.29 \pm 0.34$  mg, at 20°C it was  $0.91 \pm 0.09$  to  $81.80 \pm 0.49$  and at 25°C it was  $7.20 \pm 0.05$  to  $57.7 \pm 0.56$  (Table 3). The mean scores of vitamin C content of

radicle was high at 20°C temperature at all stages when compared to other two temperatures i.e. 15°C and 25°C (Fig. 4).

The vitamin C content at 12hrs was  $7.89 \pm 0.15$  mg (15°C)  $24.06 \pm 0.31$  (20°C) and  $24.63 \pm 0.31$  mg (25°C) and at 72hrs it was  $32.29 \pm 0.34$  mg (15°C),  $81.80 \pm 0.49$  (20°C) and  $57.7 \pm 0.56$  at 25°C. Constant increase of vitamin C content was seen at all stages in all the three temperatures (Fig. 4). The mean scores were very high when compared to the study of [3] where they reported the vitamin C content of domestically processed quinoa was  $19.38 \pm 0.28$  at 24hrs.

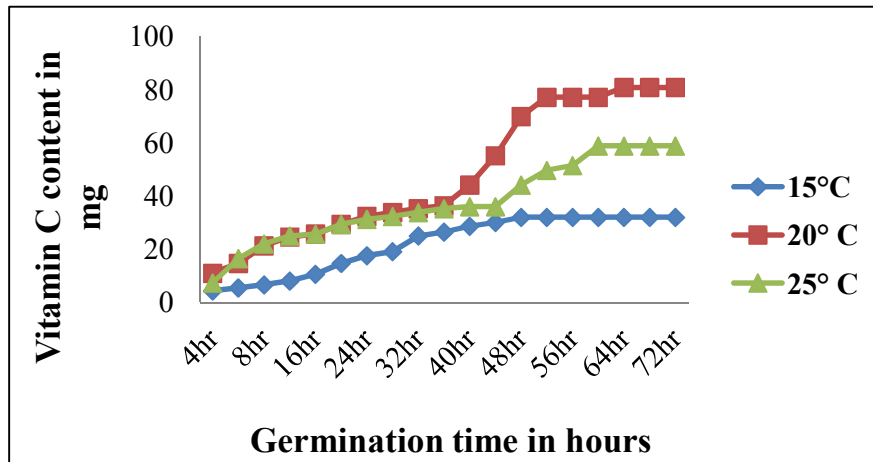


Fig. 4. Mean scores of vitamin C content of germinated quinoa seeds at three different temperatures

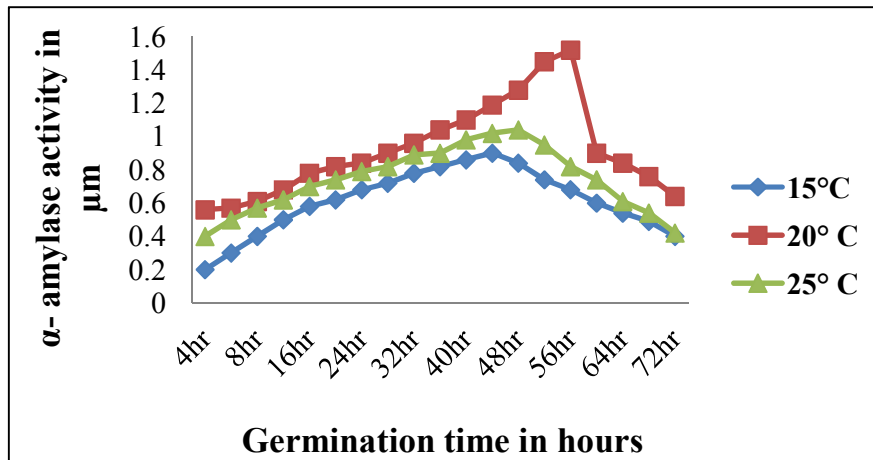


Fig. 5. Mean scores of amylase activity of germinated quinoa at three different temperatures

It was observed that during germination vitamin C was synthesized as a consequence of vitamin C biosynthesis undergone in the seeds [12]. This may be the reason for the continuous increase in vitamin C level in all the three sprouting temperature at all the time periods.

### 3.3.3 Alpha amylase activity of radicle

The mean scores of alpha amylase activity of radicles at 15°C ranged from 0.15±0.02 to 0.85±0.02 µm, at 20°C it was 0.51±0.02 to 1.48±0.02 and at 25°C it was 0.40±0.00 to 1.04±0.00 (Table 3). The mean scores of alpha amylase activity of radicle were high at 20°C temperature at all stages when compared to other two temperatures i.e. 15°C and 25°C (Fig. 5). The alpha amylase activity at 15°C reached

maximum at 44hrs (0.85±0.02µm) and from 48hrs the alpha amylase activity gradually decreased to 0.39±0.01 at 72hrs. The alpha amylase activity at 20°C reached maximum at 56hrs (1.48±0.02µm) and from 60hrs the alpha amylase activity gradually decreased to 0.66±0.01 at 72hrs. The alpha amylase activity at 25°C reached maximum at 48hrs (1.04±0.00µm) and from 48hrs the alpha amylase activity gradually decreased to 0.42±0.00 at 72hrs.

The change in α- amylase activity of quinoa grain during germination at different temperature are presented in Fig. 5. It showed that α- amylase activity was developed in germinated samples and rapidly increased up to 32hrs of imbibition, and slightly decreased after 48hrs of germination. The activity of enzymes depends on

temperature, moisture content and environmental conditions of germination [12]. It has been clearly shown in this study at 20°C temperature the amylase activity was very high showing optimum temperature factor.

The relationship between physical quality characteristics, germination speed parameter, vitamin C and alpha amylase activity at different temperatures was determined using Pearson's correlation and presented in Table 4. There was strong positive correlation between length of the radical and vitamin C content at all the temperatures studied ( $r = 0.69312$ ,  $p < 0.001$ ). The alpha amylase activity was strongly positively correlated with FGP at 15°C and 20°C ( $r = 0.69312$ ,  $p < 0.001$ ). The FGP was strongly positively correlated with length of radicle, vitamin C content and alpha amylase activity at all the temperatures studied ( $r = 0.69312$ ,  $p < 0.001$ ). MGT was negatively correlated with length of radicle and vitamin C content at all the temperatures studied ( $r = 0.69312$ ,  $p < 0.001$ ).

### 3.4 Sensory Evaluation of Germinated Quinoa

To select best accepted protocol of germination, sensory evaluation was done and the mean scores are plotted in Fig. 6 and the actual mean scores.

The mean scores of colour for germinated quinoa at 20°C in the decreasing order are  $GT_1 (7.2) > CRQ (6.70) > GT_2 (4.44) > GT_3 (3.20)$ . The mean scores of germinated quinoa at 15°C in the decreasing order are  $GT_2 (5.41) > GT_3 (5.33) > CRQ (5.00) > GT_1 (4.73)$ . The mean scores of germinated quinoa at 25°C in the decreasing order are  $C (5.00) = GT_1 (5.00) > GT_2 (4.66) > GT_3 (3.93)$ . The colour value was significantly higher ( $p \leq 0.05$ ) for  $GT_1$  at 20°C and lowest for  $GT_3$  at 20°C.

The mean scores of flavor for germinated quinoa at 20°C in the increasing order are  $GT_3 (3.62) < CRQ (5.00) < GT_2 (5.40) < GT_1 (7.26)$ . The mean scores of germinated quinoa at 15°C in the increasing order are  $GT_3, CRQ (4.73) < GT_2 (5.00) < GT_1 (5.13)$ . The mean scores of germinated quinoa at 25°C in the increasing order are  $GT_2 (3.46) < GT_3 (4.86) < CRQ (5.20) < GT_1 (5.26)$ . The flavor value was significantly lower for  $GT_2$  at 25°C and highest for  $GT_1 (7.26)$  at 20°C.

The mean scores of taste for germinated quinoa at 20°C in the decreasing order are  $GT_1 (8.00) >$

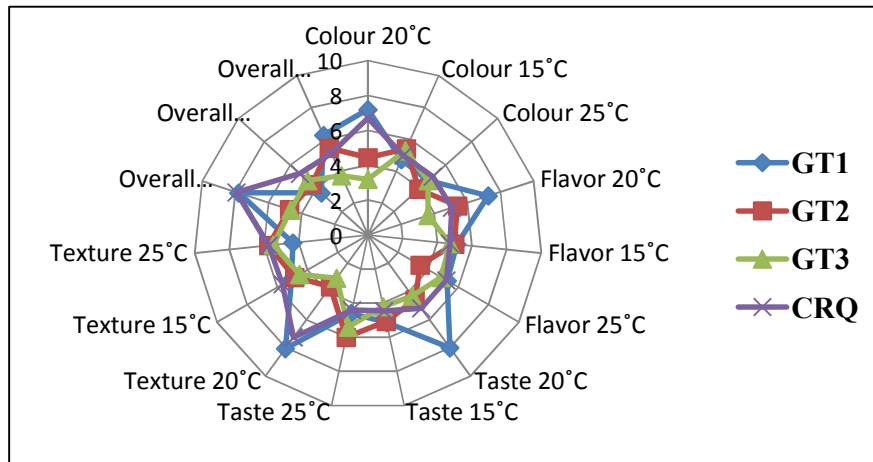
$CRQ (5.20) > GT_2 (4.60) > GT_3 (4.33)$ . The mean scores of germinated quinoa at 15°C in the decreasing order are  $GT_1 (5.13) > GT_2 (5.06) > CRQ (4.46) > GT_3 (4.20)$ . The mean scores of germinated quinoa at 25°C in the decreasing order are  $GT_2 (6.00) > GT_3 (5.40) > GT_1 (4.60) > CRQ (4.40)$ . The taste value was significantly higher for  $GT_1 (8.00)$  at 20°C and lowest for  $GT_3 (4.33)$  at 20°C.

The mean scores of texture for germinated quinoa at 20°C in the increasing order are  $GT_3 (3.06) < GT_2 (3.66) < CRQ (7.26) < GT_1 (8.06)$ . The mean scores of germinated quinoa at 15°C in the increasing order are  $GT_3 (4.53) < GT_2 (4.86) < GT_1 (5.06)$  and  $CRQ (5.66)$ . The mean scores of germinated quinoa at 25°C in the increasing order are  $GT_1 (4.33) < GT_3 (5.46) < GT_2 = CRQ (5.73)$ . The texture value was significantly low for  $GT_3$  at 20°C and highest for  $GT_1$  at 20°C.

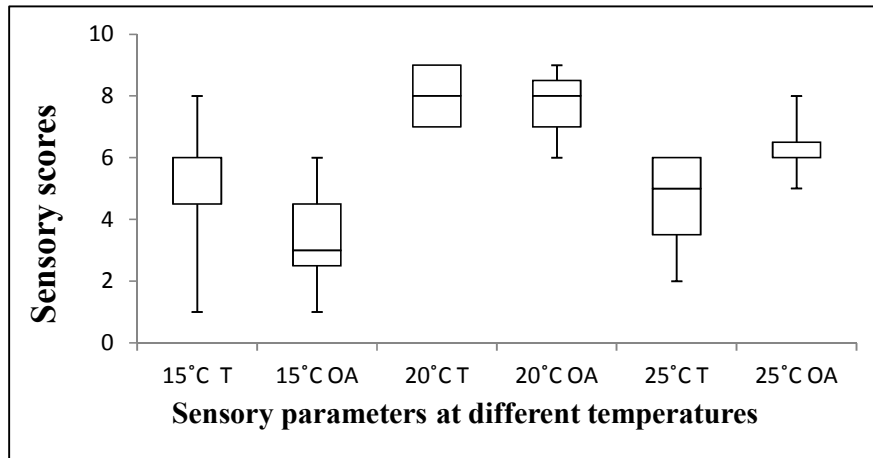
The mean scores of taste for germinated quinoa at 20°C in the increasing order are  $GT_3 (4.66) < GT_2 (4.73) < GT_1 (7.80) < CRQ (7.93)$ . The mean scores of germinated quinoa at 15°C in the increasing order are  $GT_1 (3.60) < GT_2 (4.33) < GT_3 (4.66) < CRQ (5.26)$ . The mean scores of germinated quinoa at 25°C in the increasing order are  $GT_3 (3.73) < CRQ (5.13) < GT_2 (5.46) < GT_1 (6.26)$ . The overall acceptability value was significantly lower for  $GT_1$  at 15°C and highest for C at 20°C.

Fig. 6(a) shows that the mean sensory scores are high for 4hrs of germination at all the temperatures. To select final best suitable temperature the actual scores of taste and overall acceptability given by panel members were taken and box-plot diagram was drawn. Box plot descriptive statistics are presented in Fig. 6(b). Values and maximum values of sensory scores of taste (8) and over all acceptability at 20°C incubation are very high. The 25<sup>th</sup> percentile of taste (7) and over all acceptability (7) at 20°C incubation and the 75<sup>th</sup> percentile of taste (9) and over all acceptability (8.5) at 20°C incubation is high compared to the other two temperatures 15°C, 25°C.

Sensory scores are clearly indicating that overall acceptability score was high for the 20°C temperature for 4hrs germination. The mean scores were indicating that as the length of the radicle increased the score for taste are decreasing as bitterness of grain was increasing with increase in time of germination.



**Fig. 6(a). Mean scores of sensory acceptability of germinated cooked quinoa**  
 GT<sub>1</sub> – Cooked germinated quinoa at 4hrs; GT<sub>2</sub> - Cooked germinated quinoa at 8hrs; GT<sub>3</sub>- Cooked germinated quinoa at 12hrs; CRQ – Quinoa cooked



**Fig. 6(b). Box and whisker plots displaying median, inter quartile range (box) and range (whiskers) of sensory parameters of germinated cooked quinoa at three temperatures after 4hrs of germination**  
 T – Taste, OA – Over all acceptability

Growth of plumule and mould was also seen after 12hrs, because of the above said reasons sensory evaluation of quinoa grain was done up to 12hrs only. Depending on the results of physical parameter and sensory characteristic protocol, 20°C temperature and 4hrs germination was selected for further analysis (results not reported here). The experimental sample quinoa (one of the accession line of EC series) variety was developed at Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad.

#### 4. CONCLUSION

From the study it can be concluded that the quinoa has high germinated capacity as it started

from 4hrs upon germination. The seed germinated parameters are high at 20°C compared to 15°C and 25°C. The length of the sprouts, vitamin C content and alpha amylase activity are high at 20°C compared to 15°C and 25°C. The grain germinated at 20°C for 4hrs is sensorially more accepted compared to the other temperatures and hours of germination. The germinated quinoa was incorporated in laddu and chapatti was published and the glycemic index of germinated quinoa was in process of publication.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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