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Indigenous hydropriming practice for fastening seed germination might induce reciprocal effects in chickpea (*Cicer arietinum* L.) under "on-farm" seed priming scenario

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From the vedic times, the cultivation of crops in India, were meant for sustenance of mankind. The age old practices transferred across generations have being followed by the young and the old without a second thought. The main land agriculture that has adopted several of these practices has witnessed many a kind of ups and downs from replicating these practices. The lack of a strategic scientific validation aided with the misinformation by passing the indigenous knowledge across generations have caused many a kind of deleterious effects too. One of such traditional practice is the soaking of seeds prior to germination that is being scientifically stated as the seed priming which enhances the process of germination. Though the "on- farm" seed priming technology have been pointed out as a sustainable option for the intensification of agriculture in marginal and tribal belts of the country, its recommendation needs prior standardization in and across various crop genotypes. Hence, in this study the effect of different durations of hydropriming on the germination and growth of Chickpea (*Cicer arietinum* L.) were assessed. BGD 72 variety of *Desi* and BGD 128 variety of *Kabuli* were used for the study. It was observed that there occurs wide variation in germination and seedling growth across the intervals of priming and each positive effect is sharply - duration and genotype specific.

Keywords: Chickpea, Germination, Hydro priming, "On – farm" seed priming

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In the Indian scenario, the central Indian states have a tribal population of more than 25% of the total. Among the major central Indian states, Maharashtra with 10%, Madhya Pradesh with 14% and Gujarat with 8% of the total state population are tribal[1]. Of this in Maharashtra, 40% are farmers and 45% are agricultural labourer's. The major tribal population in the states includes the *Bhil*, *Ghond* and the *Raj Ghond* communities which rely largely on the traditional agricultural practices for their sustenance.

For centuries together, Indian farmers have planned farming practices in line with the adoption of indigenous knowledge, transferred across generations. Be it the traditional knowledge engrained in the *Upanishads* or the dialectical knowledge spread by word from the great grandparents, the development of such indigenous knowledge systems, including its transmission and management, have been a matter of survival for the ones who have generated these systems. Though such systems have undergone not only cumulative experience of generations but also careful observations along with rampant trial and error, even then they changed its form by itself, as and when the time demanded it[2]. Many of these practices include the methods for enhancing shelf life of stored products, methodologies to deter pests in storage, post-harvest processing technologies or farm level application of techniques to fasten seed germination and seedling establishment etc. One of such widely adopted technology, that have gained greater interest by the farming community is "on- farm seed priming" that aids in fastening the seed germination process at field level. Being, a simple, cost effective single step solution to fasten the seed germination process many of the tribal belts of India have widely adopted this practice and is used extensively

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in major crops *viz.*, rice, groundnut, mung bean and chickpea[3].

In case of chickpea, though the scientific practices recommend for the non-soaking of seeds prior to sowing, there are several communities which adopt many of the seed soaking practices, mainly in the tribal belts of central India. Smearing seeds on turmeric and sweet flag solution, soaking seeds in curd for 24- 48 h for controlling wilt disease, soaking and drying of seeds in butter milk to reduce dry root rot diseases etc. are few among the many methods adopted by them over time immemorial. "On- farm seed priming" generally includes the overnight soaking of seeds in water and surface drying them for a certain period of time[4]. At the same time, on scientific validation of the said practice, it has been observed that the prior water soaking of seeds decreases the period of water imbibition in the soil and hence fastens the process of radicle emergence. But contrary to the general practice, it is also not clear to what extent "on –farm" seed priming can improve crop performance and what factors may delimit its outcome. Hence, a greater understanding is needed to identify the environments where it may be detrimental and where it can best be applied. But here is to be noted that the most important aspect of "on-farm" seed priming is the duration of the soaking (hours/ days), which must be calculated specifically for each crop species, as well for each variety and crop cultivar. Since, exceeding a 'safe limit' of priming/soaking might trigger the process of premature germination, which in turn could lead to wide damage of the radicle during sowing; the process needs thorough scientific validation[5]. Although farmers have a great extent of knowledge on the advantages of seed soaking in water/ nutrient solution prior to sowing[6], it is often carried out on seeds for re-sowing in order to 'catch up' with the rest of crop and is rarely used as a routine practice. In general, farmers who have used on-farm seed priming are unaware of safe limits and therefore have had varying degrees of success or failure with this method[7]. Therefore, a study was devised at the Division of Seed Science and Technology, ICAR-IARI, New Delhi to standardize the hours of hydropriming in enhancing the germination and seedling quality of chickpea.

Materials and Methods

Plant material used

Considering the tribal population undertaking different practices of agriculture in the central and north-western tracts of India, the varieties best suited for the particular location were selected for the study. BGD 72 (*Dharwad Pragati*) variety of *Desi* chickpea released in 1999 for central zone and BGD 128 (*Pusa Shubra*) variety of *Kabuli* chickpea released in 2007, for the north-western tracts of the country were opted. Seeds of both the varieties, were raised at the seed farm, Division of Seed Science and Technology, ICAR- IARI during the rabi season of 2017-18. They were cleaned thoroughly after harvest and seeds of uniform size were graded separately for conducting the experiments. All the experiments were conducted precisely, at the Division of Seed Science and Technology, ICAR-IARI during the period of 2018-19.

Standardization of hydropriming

100 seeds of each variety were selected randomly from the seed lot. Normal grade filter papers were used as the substrate and were placed in petriplate (12*5 cm diameter), in 3 replicates. The filter paper was moistened using 30 mL of single distilled water and the seeds were left to imbibe water for definite intervals of time ranging from 1 h to 7 h. After the requisite period of priming, seeds were dried back to their normal moisture content and germination tests were conducted as per ISTA, 2015.

Observations recorded

The seedling quality parameters were recorded on the day of final count of germination test. The shoot length of 10 randomly selected seedlings were measured from the point above to the cotyledon while the area specified below to the cotyledon was measured as root length. Seedling vigour index I was calculated by multiplying the germination percentage with seedling length (cm) while the Seedling vigour index II was calculated by multiplying germination percentage with seedling dry weight in gram.

Statistical analysis

The experiment was conducted in a Completely Randomized Block Design. Three replicates were maintained for each treatment. Statistical analyses were performed using the R software and the mean variance among the treatments were analysed using the Least Significant Difference (LSD) test at 0.05 level of probability.

Results

The *Desi* and *Kabuli* varieties of Chickpea BGD-72 and BGD 128 respectively were admitted to different durations of hydropriming in order to study

the variability in growth pattern, effected by differential imbibition of water. The treatment durations ranged from 1 h to 7 h while non-treated seeds were taken as the control. 100 seeds in 3 replicates were soaked in 30 mL of single distilled water and were dried back to normal moisture content. The percentage of Seed Germination, Shoot length (cm), Root length (cm), Seedling Length (cm), Shoot Dry Weight (SDW), Root Dry Weight (RDW) and Seedling Dry Weight (SdDW) were observed on the 8th day of germination test. It was observed that the priming of seeds with single distilled water, have significant effects on the germination and seedling growth parameters of chickpea seeds (Table 1 and 2).

In case of *Desi*, soaking of BGD-72 variety of chickpea seeds have shown slightly improved percentage of germination, up to 3 h of priming while it reduced later on. Highest percentage of germination was observed at 2 h of priming compared to all other treatments. The greatest improvement in the length of shoot was observed at 1 h of priming while it decreased at 2 h of priming. Further, the same pattern of increase-decrease of shoot length was observed in the durations of priming that followed. At the same time, the least length of shoot growth was observed in control (without any treatments) and was found to be on par with 6 h of priming (Fig. 1.). The root length (cm) of the seedlings were recorded on the 8th day of germination test. It was observed significantly that, the maximum growth of root was observed in 1 h of germination and was found to be on par with 3 h, 6 h and 7 h of priming. While the least was observed on control (non-treated seeds), and was followed by 2 h of priming and further by 4 h of priming. Consequently, maximum seedling length was observed in 1 h of priming in BGD 72 and it was followed by 7 h and 6 h of priming. At the same time, 2 h and 5 h of priming, have shown significantly on par seedling growth while the least was observed for control (non-treated seeds) (Fig. 2) Meanwhile, the dry weight of shoot, root and seedlings were taken separately on a random sample of 10 seedlings per replicate. It was observed that 1 h of priming have shown significantly better shoot dry weight and was

Table 1 — Effect of hydro priming duration on the germination and growth parameters of desi chickpea. Abbreviations: H- Hours of priming, SL- Shoot Length (cm), RL- Root length (cm) SdL – Seedling Length (cm), SDW- Shoot Dry Weight (gm), RDW- Root Dry Weight (gm), SdDW- Seedling Dry Weight (gm).

Treatments	Germination	SL	RL	SdL	SDW	RDW	SdDW
Control	93	11.665	17.955	29.62	0.255	0.2125	2.4645
1 h	95	14.3	22.13	36.43	0.595	0.3085	2.5175
2 h	96	12.485	20.79	33.275	0.525	0.2815	2.4985
3 h	94	13.16	22.16	35.32	0.341	0.2085	2.831
4 h	93	12.075	20.395	32.47	0.82	0.3465	3.14
5 h	91	12.035	21.445	33.48	0.205	0.279	2.143
6 h	90	11.8	22.335	34.135	0.705	0.2035	0.904
7 h	89	13.65	22.26	35.91	0.48	0.2935	0.9875
C.D.	0.023	1.21	1.34	2.23	0.674	0.085	0.319
SE (m)	0.121	0.657	1.205	1.385	0.148	0.025	0.094
SE (d)	0.016	0.929	1.704	1.958	0.21	0.035	0.133
C.V.	5.44	7.347	8.046	5.789	42.793	13.233	5.905

Table 2 — Effect of hydro priming duration on the germination and growth parameters of kabuli chickpea. Abbreviations: H- Hours of priming, SL- Shoot Length (cm), RL- Root Length (cm) SdL – Seedling Length (cm), SDW- Shoot Dry Weight (gm), RDW- Root Dry Weight (gm), SdDW- Seedling Dry Weight (gm).

Treatments	Germination	SL	RL	SdL	SDW	RDW	SdDW
control	90	10.895	16.77	27.665	0.31	0.27	2.23
1 h	93	10.91	15.805	26.715	0.56	0.33	2.45
2 h	92	12.54	19.535	32.075	0.54	0.26	2.19
3 h	91	11.025	20.285	31.31	0.41	0.31	2.21
4 h	91	11.22	21.215	32.435	0.69	0.32	2.76
5 h	87	10.57	17.8	28.37	0.32	0.29	3.12
6 h	89	12.12	18.445	30.565	0.43	0.54	1.13
7 h	87	12.905	19.6	32.505	0.51	0.48	1.11
C.D.	0.689	1.16	1.87	1.45	0.867	0.574	0.932
SE (m)	0.115	0.695	1.102	1.737	0.176	0.234	0.068
SE (d)	0.125	0.982	1.558	2.457	0.32	0.398	0.231
C.V.	7.78	8.525	8.34	8.134	8.9	7.86	4.32

found to be on par with 2 h of priming. While, the highest shoot dry weight was observed on 4 h of priming, followed by 6 h of priming, while the least was on control and was found to be on par with 5 h of priming. Considering the root growth parameters, root dry weight of 10 random seedling's per replicate were estimated using oven dry method. It was observed that the highest improvement in root dry weight happens during 4 h of priming, followed by 1 h of priming while the least was in control and was found to be on par with 3 and 6 h of priming, respectively. Seedling dry weight of 10 randomly selected seedlings per replicate were estimated using the oven dry method. It was observed that the maximum seedling dry weight is acquired when the seeds are kept for 5 h of priming, which shows a steady decline after wards.

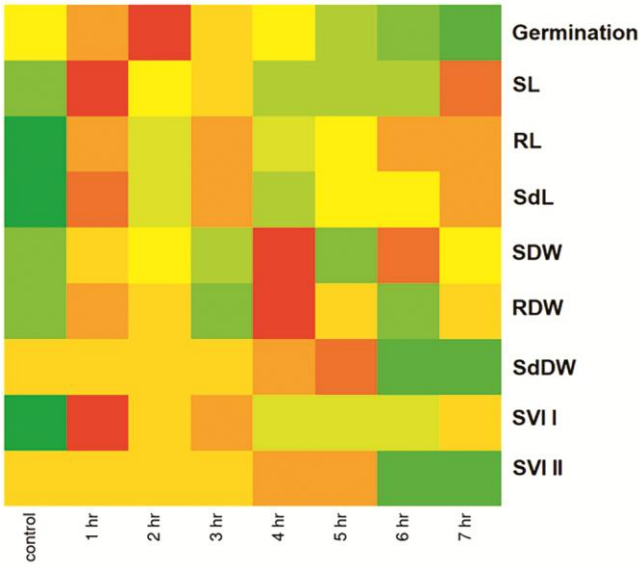

Fig. 1 — Heat map showing the variation in the germination potential and seedling growth parameters across different durations of priming in desichickpea. Abbreviations: control – non-treated seeds, H -Hours of priming, Germination - Percentage of Germination. SL- Shoot Length (cm), RL- Root Length (cm) SdL - Seedling Length (cm) SDW - Shoot Dry Weight, RDW - Root Dry Weight SdDW - Seedling Dry Weight, SVI I - Seedling Vigour Index I and SVI II - Seedling Vigour Index II. The colour gradient from green- yellow- red represents low to high values.

At the same time in case of *Kabuli* variety BGD 128, it was observed that the highest percentage of germination was in 1 h of priming followed by 2 h of priming while the least was in 5 h of priming and was found to be on par with 7 h of priming. Here, it has to be taken note of that the increased durations of priming, significantly reduces the germination percentage in Kabuli and the effect reduces gradually to the level that the control (non-treated seeds) starts performing better. The significant reduction in the germination percentage was observed from 5 h of priming and continued thereafter (Table 2).

The shoot length (cm) was observed on the 8th day of germination test and it was found that the significant improvement in shoot length, have happened in 2 h of priming. This was on par with 6 h and 7 h of priming, while the least was for control (Fig. 3). With respect to root length, significant improvement was found in 4 h of priming, followed by 3 h of priming while, the least was found in 1 h of priming followed by control. Here it is to be observed

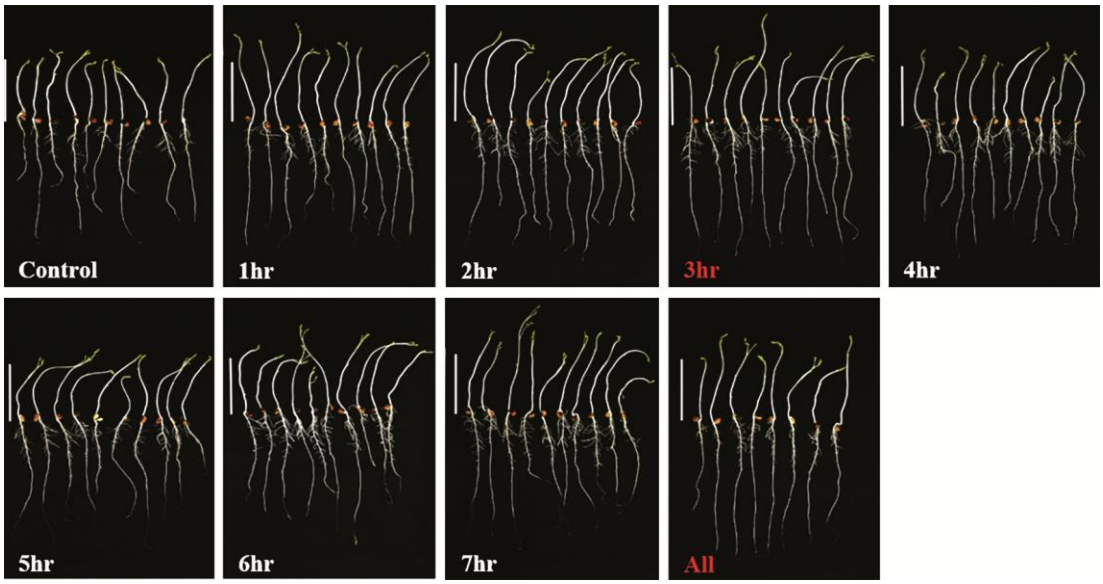

Fig. 2 — The above pictorial representation indicates the variation in the seedling growth parameters of *desi* chickpea across various durations of Hydropriming. Abbreviations: Hr - Hours of priming and Control indicates non-treated seeds.

that though less hours of priming have significantly improved the percentage of germination, its effect on the physiological growth of seedlings stands opposite

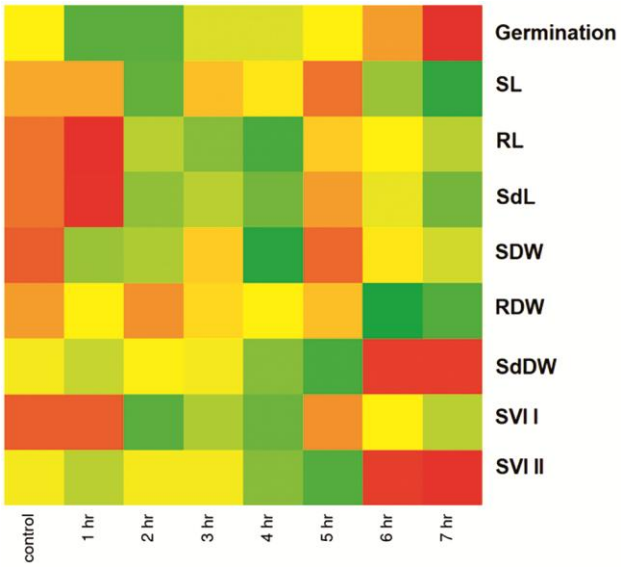

Fig. 3 — Above heat map indicates the variation in the germination potential and seedling growth parameters across different durations of priming in Kabuli Chickpea. Abbreviations: control – non-treated seeds, H -Hours of priming, Germination - Percentage of Germination. SL- Shoot Length (cm), RL- Root Length (cm) SdL - Seedling Length (cm) SDW - Shoot Dry Weight, RDW - Root Dry Weight SdDW - Seedling Dry Weight, SVI I - Seedling Vigour Index I and SVI II - Seedling Vigour Index II. The colour gradient from green- yellow- red represents low to high values.

(Fig. 4). On a combined analysis of the seedling length, it was observed that 4 h of priming have significantly improved the seedling growth rate and was found to be on par with 2 h of priming and with 7 h of priming, while the least growth in seedling rate was observed in 1 h of priming followed by control (non-treated seeds). Further, on analysis of dry weight of seedling parts in separation and in combination, it was observed that in 4 h of priming, maximum improvement in shoot dry weight happens, followed by 1 h of priming which is on par with 2 h of priming, while the least was for control. On an extended observation of root dry weight of seedlings, it was observed that maximum root dry weight was for 6 h of priming followed by 7 h of priming while the least was for control, which is on par with 2 h of priming and further by 5 h of priming. Seedling dry weight of 10 randomly selected seedlings per replicate were estimated using the oven dry method. It was observed that the maximum seedling dry weight is acquired when the seeds are kept for 5 h of priming, which shows a steady decline after wards.

Discussion

The technology of on-farm seed priming, is under wider acceptance among majority of farming communities in India, who replicate and co-opt traditional farming practices along with conventional agriculture. Though the practice have been transferred

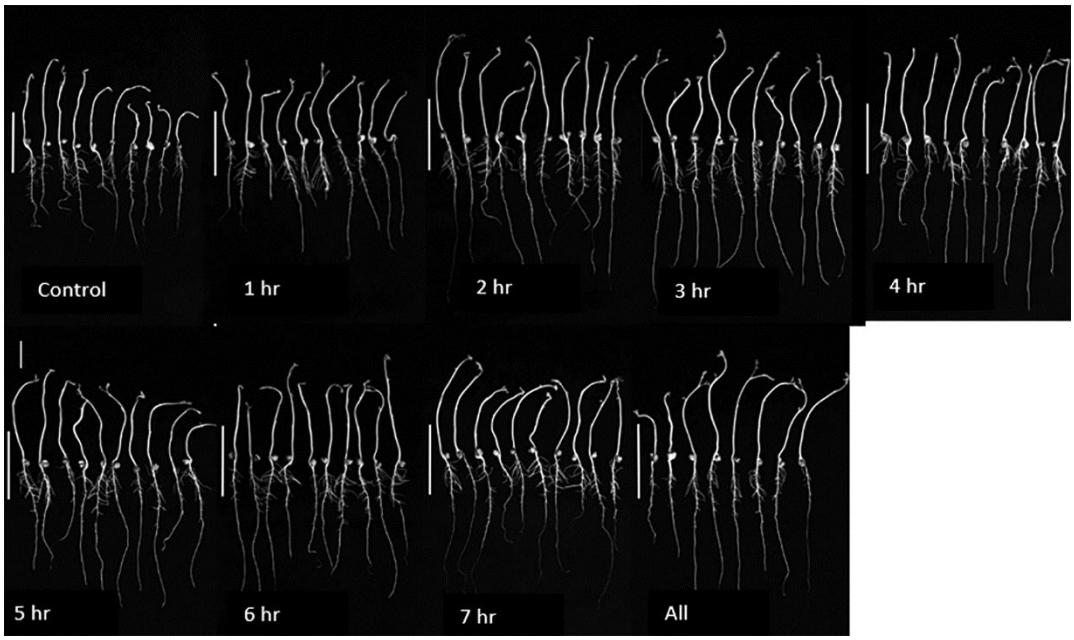

Fig. 4 — The above pictorial representation depicts the variation in the seedling growth parameters of Kabuli chickpea across various durations of hydropriming. Abbreviations: H - Hours of priming and Control indicates non - treated seeds.

across generations, an umbrella application of the method across various crops in different environmental and climatic conditions have incurred severe negative effects on the farmers *viz.* decline in germination, scattered seedling growth, reduction in yield potential etc[8]. Hence an immediate study was undertaken in order to scientifically validate the process of on-farm seed priming, by adopting the principle of seed hydropriming and a lab experiment to standardize the duration of hydropriming hours in chickpea was conducted.

Seed germination starts with the imbibition- *i.e.*, the physical uptake of water by mature seeds[9]. Water is found necessary for the reactivation of metabolic processes, since it promotes the biochemical reaction within the cells. Along with it, water also stabilizes the macromolecular structures and also acts as a mediator/ reactant in several biochemical processes[10]. The reactivation of metabolism along with the generation of adequate turgor pressure, by increased water content, leads to the growth of embryo and thereby to the protrusion of radicle out of the seed coat, which is stated as the endpoint of germination. Here, in this study, different durations of hydropriming were given so as to analyse its effects on the seed germination percentage and seedling growth characters. From the results obtained, it was found that 2 h of priming have significantly improved germination percentage in *Desi* chickpea, while in *Kabuli*, 1 h have shown greater percentage of germination. But when the percentage germination was compared with the seedling vigour index, it could be concluded that 3 h of priming in *Desi* have significant improvements when compared to control, while in *Kabuli*, 4 h was found to be the best.

Thus, the study indicates that the hydroprimed seeds have shown significantly better germination when compared to the non-treated seeds (control). The better germination percentage can be attributed to the increased percentage of imbibition that triggers the so called – "pre germinative metabolism". The rapid increase in the uptake of water might have turned in, the series of biochemical events within the seeds such as the use of reserve compounds (carbohydrates, lipids and proteins), activation of anti-oxidant enzymes and subsequently the sensing of DNA damage and its repair. The further decline in germination percentage after a certain period of time, might be attributed to the over-imbibition of water, leading to stress within the biological compartments.

Our results have proved that the different durations of hydropriming treatments have significantly improved the percentage of seed germination. This is in agreement with the findings of Forti *et al.*[11], whose focussed research on the hydropriming effects on *Medicago sativa*, and reported that the various genes including OGG 1 (8-Oxoguanine DNA glycosylase) and FPG (Formamidopyrimidine –DNA glycosylase) expresses differentially during the alternative durations of hydropriming effecting differential pattern in Base Excision Repair (BER). Concomitantly they also observed significant up regulation in the Super oxide dismutase gene encoding ROS scavengers which have resulted in better percentage of germination. This is also in agreement with the recent literatures citing that hydropriming mainly improves the percent of germination owing to increased uptake of water, and by mitigating the negatives effects of temperature on germination. The beneficial effects of seed priming have been attributed to the activation of various enzymes in the embryonic tissues that are being used for the rapid germination of seeds and for the mobilization of compounds including free amino acids, proteins and soluble sugars from the various organs of storage[12]. The rapid mobilization of seed metabolites to the seed embryo, ensures the rapid germination of seeds. Moreover, the reduction of metabolite leakage and the repair of deteriorated tissues, increased RNA repair and the improvement in protein synthesis have added advantage on the germination of seeds[13].

The increase in germination percentage of chickpea seeds recorded in the present study, might be also due to the increase in metabolism prior to the second phase of germination and the production of sugars that are being used in the synthesis of proteins during the different phases of germination[14]. The increase in seed performances owing to hydropriming can hence be attributed to the repair and accumulation of nucleic acids, increased protein synthesis, membrane repair and due to the improved anti-oxidant system. Akinola *et al.*[15] have reported that hydropriming at different durations have increased the percentage of germination in sunflower. The present findings are in correlation with the results of Thornton and Powell, 1992 in Brassica[16], Fujikara *et al*., 1993 in Cauliflower[17], Diniz *et al*., 2009 in Sweet pepper[18] and Caseiro *et al*., 2004 in Onion[19].

As Kloppenburg (1991) has indicated, "the local knowledge or the *mutable immobile* are relatively malleable knowledge, that are being developed in order

to cater the needs of the local population"[20]. Hence, only a comparative advantage from the same can be obtained from the local community while a wider extend of application of the same without a scientific validation might induce reciprocal effects[21-24].

Conclusion

In the developing countries, tackling the problem of yield reduction caused by both natural and the socio economic constraints require simple single step solutions which are economic and cost effective. Hence forth, majority resort upon the locally available traditional knowledge and the umbrella application of the same points out to the negative studies as well. Hence, in this study a quantification of such an indigenous practice was carried out and it was observed that the procedure of on-farm seed priming can be made more effective, if it is standardized for each crop and genotype by taking the hours of priming as a dependent factor. The hydropriming practice attenuates the negative effect incurred on the planting conditions and facilities the rapid crop establishment in the field. This can also be visualized as a starting point for intensification of traditional agriculture with smart use of scientific validation across the various traditional farming belts.

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Conflict of Interest

Authors hereby declare that, there are no competing conflict of interest with regards to the specific work undertaken.

Authors' Contributions

VGD- Original draft preparation, writing, review and editing, AKMB- Conceptualization, funding acquisition and supervision, CB- Review and editing, A - Statistical analysis and proof reading.

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