MONITORING SEED PROTEIN CONTENT (SPC) OF FOUR BELL PEPPER CULTIVARS HARVESTED AT DIFFERENT FRUIT AGES TO ASSESS THEIR VIABILITY

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ABSTRACT
To investigate the appropriate time of harvest for four cultivars of bell-pepper (Capsicum annuum), a mass transplanting of "Dan Datsa", "Dan Charkawa, "Dan Damaso", and "Dan Boko" cultivars of "tatashe" was carried out in the 2018 dry season at 75 × 50 cm inter-row and intra-row spacings respectively. Each cultivar was transplanted in dedicated blocks measured at 108 m². The flowers were tagged at full anthesis, and the fruits were harvested at 25, 30, 35, 40, 45, 50 and 60 days after anthesis (DAA). Seeds were extracted, processed, packaged, and stored in an incubator set at 35 °C and 75% relative humidity (RH) to accelerate the ageing process of the seeds. Samples were collected every week for viability test and monitoring of seed protein content. The seeds of "Dan Damaso" cultivar recorded significantly higher (36.57 - 1.57%) seed germination percentage (SGP) and significantly higher seed protein content (23.78 – 18.24%) which was across the storage period. While other cultivars recorded germination ranging between 0.50 - 31.71% germination and 16.31 – 21.34% SPC. Fruits harvested at 50 DAA recorded significantly higher SGP and SPC across the storage period. Across the storage period, irrespective of harvesting stages, SGP recorded increase in values within the first 2-3 weeks of storage while the SPC values reduces as the storage period progressed. From this study, we can conclude that studying the seed protein contend of bell-pepper seeds can be used to monitor the viability and vigour of bell pepper seeds during storage.
Keywords: *ageing process, full anthesis, seed protein content, storage period, viability test*

INTRODUCTION

Globally, pepper is a very important vegetable crop for both fresh and processed food markets (Airaki *et al.*, 2012). Over 200 million Nigerians irrespective of their socio-economic status use pepper extensively for either, colouring, flavouring, soup making, or stew thickening (Gates *et al.*, 2007). However, seed production in Nigeria has been undermined by the incidence of pests, diseases, high cost of inputs (pesticides, herbicide, and fertilizer), land over-utilization, and poor-quality seeds (Kudi *et al.*, 2008). Also, majority of seed companies in Nigeria place a high priority on the production and distribution of cereals and legumes leaving the small-holder vegetable farmers to source seeds by themselves. These small-holder farmers mostly collect seeds and fruits that have been left to weather on the field which are of poor quality (Ibrahim *et al.* 2017).

Oladiran and Kortse (2002) stated that the production of high-quality vegetable seeds is dependent on genotype and the appropriate time of harvest. During the fruit maturation process, seeds go through physical, biochemical, and physiological changes which are influenced by genetic (Makhaye *et al.*, 2021) and environmental (Kurukalasuliya *et al.*, 2013) factors. Ibrahim *et al.* (2017) reported that both mass and physiological maturity of seeds were attained in all four genotypes of pepper used at 52 days after anthesis (DAA). Vigidal *et al.* (2009) reported that the vigour of sweet pepper seeds extracted from fruits that were harvested at 60 or 70 days after anthesis was higher than those obtained from fruits harvested at 40 or 50 days after anthesis. A vigorous lot have both the high percentage of viable seeds in the sample and can also produce normal seedlings under in less than optimum or adverse conditions like what is obtainable on the field (ISTA, 2021). Using a vigorous seed not only results in fast and uniform

germination but also assists in competing with weeds on the field for water, light, and soil nutrients (Cheng *et al.*, 2015).

According to Vandamme *et al.* (2016), seed weight and seed nutrient content affect plant growth and seedling state. Previous research related seedling vigour to seed size and its protein concentration (Han *et al.*, 2014). Snider *et al.* (2016) listed seed mass, total oil, and protein calorie content to be associated with early seedling vigour of cotton. Wen *et al.* (2018) used the absolute protein content (APC) of wheat seed to evaluate the plant's dry matter and assess seed vigour. In this research, we assess the variation in seed protein content among four bell pepper cultivars and monitor the changes in protein content as the seed ages. Secondly, the relationship between fruit age, seed protein content and their influence on seed vigour was also monitored as the seed aged. Therefore, the objective of this research is to evaluate viability and vigour by measuring their seed protein content.

MATERIALS AND METHODS

Seeds from the four cultivars of bell-pepper used ("Dan Datsa", "Dan Charkawa", "Dan Damaso" and "Dan Boko") and harvested at seven different fruit maturation stage (25, 30, 35, 40, 45, 50 and 55 days after anthesis) in a factorial combination of 4 × 7 arranged in a completely randomized design (CRD) and replicated four times. Twenty-eight different seed lot of bell-pepper were placed in plastic containers with perforated lids which were stored in an incubator set at 35°C and 75% relative humidity to accelerate the ageing process of the seeds. Samples were taken every week, or every order week and the following was evaluated.

Seed germination percentage (SGP): The seed germination percentage was determined by counting 50 seeds from each lot and placing them on a moistened paper towel. They are observed for two weeks, and germination count is conducted every-order-week and expressed in percentage.

Seed protein content (SPC): About 0.5g of sample was taken and added into the digestion tube where also 20ml of concentrated sulphuric acid was added. One selenium tablet was added as catalyst. The content in the tube was heated at a temperature of 35°C for 6 hours until a clear digest was achieved, that is a clear solution. This solution was poured into a standard flask and made up to 100ml (Wen *et al.*, 2018). The data collected on all the parameters were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) and where significant differences among the treatments are obtained, means were separated using the Duncan Multiple Range Test (DMRT) at 5% probability unless otherwise stated.

RESULTS AND DISCUSSION

Figure 1 shows that seeds extracted from "Dan Damaso" cultivar fruits did not only record significantly higher seed germination percentage (SGP) during storage but also broke its dormancy faster by attaining its highest SGP (36.57%) at an early 2 weeks after storage (WAS). Other cultivars, "Dan Datsa", "Dan Charkawa" and "Dan Boko" attained their highest SGP values (20.29, 18.5 and 17.0% germination) at a later 3 WAS respectively. Seeds extracted from the fruits of "Dan Charkawa" cultivar recorded significantly lower SGP values which ranged between 0.5 and 17% germination during the 8 weeks of storage although the values were statistically at par with those of "Dan Datsa" between 4 WAS up until the end of storage (8 WAS).

Dormancy has been reported to be present in freshly harvested seeds of *Solanum spp* (Yogeesha *et al.* 2008) and this dormancy was also reported by Oladiran and Kortse (2002) to vary among pepper types. Ibrahim et al. (2018) also observed variation in viability among pepper genotypes. The variation in SGP and dormancy levels among the four cultivars of bell pepper used could be ascribed to their genetic make-up. Ramirez-Rosales *et al.* (2004) also reported variations in germination percentages among tomato genotypes which they attributed to the low germination in some cultivars due to their high lycopene content. High lycopene content is also attributed to high ABA content (Seyed and Naser, 2012). Abscisic acid (ABA) and gibberellins (GA) are well-known phytohormones that are involved in regulating seed

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germination. The two hormones operate in opposite manners, as the ABA inhibits germination while the GA promotes the biological process of germination (Nambara *et al.*, 2010).

Figure 2 shows the effects of fruit harvesting stages on the SGP of bell pepper plants. Seed extracted from fruits that attained 50 days after anthesis (DAA) recorded significantly higher SGP values during storage with values ranging between 2.87 and 43.25% at 8 and 2 WAS respectively. Seeds extracted from fruits harvested at 25 DAA produced seeds with significantly lower SGP values during the periods of storage. These values ranged from 0.00 to 7.38 recorded at 8 and 3 WAS respectively. This suggests that at 50 DAA maximum seed filling, sufficient nutrient absorption of soil nutrient which is translocated into the seeds in the fruit has occurred. This process might have conferred superiority in the capacity of these seeds over those of other ages. This was similar to the findings of Demir and Samit (2001) who reported that harvesting tomato when they are fully red, and firm (70DAA) gave the maximum seed quality regardless of the seed extraction method used.

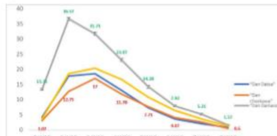

Figure 1: Effects of cultivar on seed germination percentage of bell pepper plants

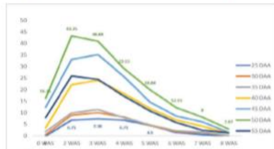

Figure 2: Effect of fruit age on seed germination percentage of bell pepper plants

Table 1 shows the interaction effects of cultivar and fruit age on the SGP of four bell pepper harvested at different fruit ages. Seeds extracted from the fruits of "Dan Damaso" cultivar and harvested when the fruits attained 50 days after anthesis produced seeds with significantly higher SGP values across the period of storage. These values ranged from 73.50% obtained at 2 WAS and 4.00% germination obtained at the end of storage (8 WAS). Irrespective of cultivar, seeds extracted from fruits that attained just 25 DAA recorded the lowest SGP values across the 8 weeks of storage.

Figure 3 shows the effects of cultivar on the seed protein contents (SPC) of four bell-pepper plants. "Dan Damaso" cultivar of bell-pepper produced seeds with significantly higher SPC during the storage period with values ranging between 23.78% recorded at the onset of storage (0 WAS) and 18.24% protein recorded at end of storage (8 WAS). Seeds extracted from the fruits of "Dan Boko" cultivar recorded the least SPC with values ranging from 19.13% protein at 0 WAS and 16.31% protein at 8 WAS. Adepoju *et al.* (2019) observed varying types of protein bands among "Ijosi" and "Sombo" varieties of pepper. According to Gao *et al.* (2016), the content of storage proteins is one of the main determinants of seed longevity. This statement is supported by the fact that the biggest differences between seeds with high and low quality are seen in their storage proteins (Sathish *et al.*, 2015). The superiority of "Dan Damaso" cultivar in seed protein content could be as a result of its ability to assimilate more nutrient required for protein production and also has the capacity to translocate more of this nutrient into the seed.

In figure 4, at 2 WAS, seeds extracted from fruits that attained 40 DAA recorded significantly high SPC value (21.58% protein), while at 0, 4, 6 and 8 WAS seeds extracted from fruits that attained 50 DAA recorded significantly higher SPC values with 25.51, 22.53, 21.75 and 20.08% protein. Fruits that attained 25 DAA before harvest produced seeds with significantly lower SPC values across the period of storage. These values ranged between 16.91 and 14.99% protein recorded at 0 and 8 WAS. The interaction between cultivar and fruit age on the seed protein content of bell-pepper plant is shown on Table 2. The table shows that fruits of "Dan Damaso" cultivar that attain 50 DAA produced seed that contains significantly higher SPC (27.28, 24.34 and 20.18% protein) recorded at 0, 2 and 6 WAS respectively. Fruits of "Dan Boko" cultivar that were harvested when the fruits that attained 25 DAA recorded the lowest SPC (15.26, 14.14 and 14.64% protein) across the storage period.

This could be tied to the fact that matured seeds have more storage reserves, desiccation, dormancy induction, better seed coat formation, and protective chemical synthesis (Naoto *et al.*, 2016). Zhang, *et al.* (2020) supported this claim by postulating that during the aging process in seeds, the changes include alterations in membrane, protein composition, disruption of the nuclear envelope, protein degradation, decreases in lipid content, oxidative stress, and decreases in mRNA translation and DNA replication capabilities.

Table 1: Interaction effects of cultivar and fruit harvesting stage on the seed germination percentage of bell pepper plants stored for eight weeks.

Interaction		Storage period (Weeks)								
Cultivar	Fruit Age (DAA)	0	1	2	3	4	5	6	7	8
Datsa	25	0.00 o	3.50 mno	8.00 op	7.00 op	4.00 p	1.00 l	0.00 n	0.00 j	0.00 f
Charkawa	25	0.00 o	2.50 op	6.00 q	4.50 op	3.50 p	3.50 k	1.00 m	0.00 j	0.00 f
Damaso	25	0.00 o	4.00 mn	10.00 n	10.00 no	9.50 lm	6.50 ghi	3.50 k	2.00 h	0.00 f
Boko	25	0.00 o	0.00 r	3.00 r	8.00 op	10.00 l	7.00 gh	1.00 m	0.00 j	0.00 f
Datsa	30	2.00 lm	6.00 l	13.00 m	8.00 op	5.50 o	3.00 k	0.00 n	0.00 j	0.00 f
Charkawa	30	0.00 o	3.00 no	7.50 p	9.00 no	5.50 o	5.00 j	3.00 k	2.50 h	0.50 ef
Damaso	30	0.00 o	4.50 m	10.00 n	14.00 k	13.00 j	6.50 ghi	3.00 k	2.00 h	0.00 f
Boko	30	0.00 o	1.00 qr	5.50 q	9.00 no	8.50 mn	3.00 k	1.50 lm	0.50 ij	0.00 f
Datsa	35	2.25 klm	7.75 k	15.00 l	9.00 no	5.50 o	3.50 k	1.00 m	0.50 ij	0.00 f
Charkawa	35	1.50 mn	3.75 mn	9.25 no	10.50 m	5.50 o	5.50 ij	3.00 k	2.50 h	0.50 ef
Damaso	35	1.00 kl	4.50 m	10.00 n	16.50 j	13.00 j	6.00 hij	3.00 k	2.00 h	0.00 f
Boko	35	1.10 n	1.50 pq	5.50 q	9.50 mn	7.50 n	3.50 k	1.50 lm	1.00 i	0.00 f
Datsa	40	3.50 j	8.50 jk	12.00 m	12.00 l	11.50 k	9.50 g	4.50 j	2.50 h	0.00 f
Charkawa	40	5.00 i	6.00 l	8.00op	30.00 f	10.00 l	7.00 gh	5.00 ij	3.50 g	0.50 ef
Damaso	40	3.00 jk	23.00 d	47.50 c	43.00 c	27.50 c	19.50 c	8.00 ef	5.00 de	2.00 bc
Boko	40	2.50 kl	9.00 ij	21.00 i	27.00 g	24.00 ef	15.00 d	9.00 de	5.50 d	1.50 cd
Datsa	45	5.00 i	12.50 g	23.00 h	27.00 g	19.50 h	10.50 f	5.00 ij	4.00 fg	1.50 cd
Charkawa	45	6.00 h	10.50 h	16.50 jk	21.50 i	21.50 g	13.50 e	6.50 g	4.50 ef	1.50 cd
Damaso	45	28.50 b	44.00 b	57.00 b	52.00 b	36.00b	17.50 c	11.50 c	9.00 b	2.50 b
Boko	45	10.00 e	20.00 f	35.00 e	31.50 e	25.50 d	16.50 c	11.00 c	6.50 c	1.50 cd
Datsa	50	11.00 d	20.00 f	30.50 f	37.00 d	25.00 de	15.00 d	8.50 ef	5.50 d	2.50 b
Charkawa	50	7.00 g	10.00 hi	26.50 g	10.00 no	23.00 f	14.00 de	8.00 f	5.00 de	0.50 ef
Damaso	50	38.00 a	58.00 a	73.50 a	61.00 a	42.50 a	29.00 a	16.50 a	12.00 a	4.00 a
Boko	50	9.00 f	21.50 e	42.50 d	36.00 d	26.00 d	21.50 b	15.00 b	9.50 b	4.50 a
Datsa	55	5.50 hi	12.50 g	22.50 h	29.50 f	20.00 h	10.50 f	6.00 gh	2.00 h	1.50 cd
Charkawa	55	2.00 lm	6.00 l	15.00 kl	14.00 k	13.50 j	5.50 ij	2.00 l	0.50 ij	0.00 f
Damaso	55	21.50 c	34.00 c	48.00 c	25.50 i	20.00 h	17.00 c	9.50 d	4.50 ef	2.50 b
Boko	55	2.50 kl	11.00 h	17.50 j	21.00 i	15.00 i	9.50 f	5.50 hi	2.50 h	1.00 de
±SE		0.74	1.08	1.14	1.31	1.17	1.18	0.78	0.71	0.42

Any two means within each column not sharing a letter differ significantly from each other by LSD at 5 % probability level.

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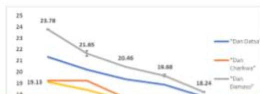

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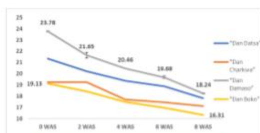

Figure 3: Effects of cultivar on seed protein contents of four bell pepper plants

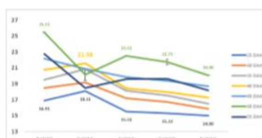

Figure 4: Effects of fruit age on seed protein content of bell pepper plant

Table 2: Interaction effects of cultivar and fruit harvesting stages on the seed protein content of bell pepper plants

Interaction		Storage periods (weeks)		
Cultivar	Harvesting stages	0	4	6
Datsa	25	17.49 t	15.28 v	14.86 y
Charkawa	25	16.26 v	15.34 u	15.12 w
Damaso	25	18.64 r	17.29 r	16.28 s
Boko	25	15.26 w	14.14 x	13.64 z
Datsa	30	18.89 q	17.32 r	15.32 v
Charkawa	30	17.40 u	16.34 t	16.09 u
Damaso	30	21.41 h	19.88 i	17.69 j
Boko	30	16.25 v	15.19 w	14.29 z
Datsa	35	19.76 o	18.12 o	17.35 n
Charkawa	35	17.83 s	16.92 s	16.24 t
Damaso	35	22.62 g	18.63 l	17.49 l
Boko	35	17.79 s	18.92 j	14.93 x
Datsa	40	20.81 k	18.44 n	17.44 m
Charkawa	40	18.65 r	17.31 r	16.86 q
Damaso	40	24.26 f	20.66 d	18.27 g
Boko	40	19.23 p	17.53 q	16.58 r
Datsa	45	21.34 i	20.08 g	19.85 e
Charkawa	45	20.45 l	18.58 m	17.26 o
Damaso	45	26.84 b	21.85 b	19.93 c
Boko	45	20.08 m	18.84 k	17.67 k
Datsa	50	26.24 c	24.34 a	20.04 b
Charkawa	50	24.25 f	21.29 c	20.19 a
Damaso	50	27.28 a	24.34 a	20.18 a
Boko	50	24.25 f	20.15 f	19.89 d
Datsa	55	24.82 e	20.01 h	19.83 f
Charkawa	55	19.83 n	18.14 o	17.96 h
Damaso	55	25.93 d	20.55 e	17.83 i
Boko	55	21.04 j	17.65 p	17.17 p
±SE		0.04	0.03	0.01

Any two means within each column not sharing a letter differ significantly from each other by LSD at 5 % probability level.

CONCLUSION

The study further established that seed protein content has a direct correlation with seed viability, vigour and longevity. The cultivar "Dan Damaso" seeds extracted from fruit that attained 50 days after anthesis before harvest produced seeds that were more viable and contain higher percentages of seed protein content. Therefore, monitoring seed protein content during storage can be used to monitor bell pepper seed viability and vigour.

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