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SCREENING OF COMMON BEAN CULTIVARS (*PHASEOLUS VULGARIS* L.) FOR DROUGHT TOLERANCE - 1

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ABSTRACT: *Drought is increasingly becoming a serious challenge reducing common bean productivity in Lesotho. A study was conducted in Lesotho with the object of (1) verifying the differences among common bean cultivars in response to induced water deficit stress, (2) evaluating common bean cultivars against different concentration levels of Polyethylene glycol which induces drought stress in germinating seed and seedling growth and (3) identifying cultivars of common beans tolerant to drought stress and rank top performing accordingly. Complete Randomized Design with three replications and 28 treatments were employed in the laboratory experiment. Treatments were seeds of common bean cultivars obtained from Department of Agricultural Research in Maseru, Lesotho and four different concentrations of PEG-6000. Parameters measured were germination percentage, germination stress index, plumule length, radicle length, plumule fresh and dry weight and radicle fresh and dry weight. Significant differences (P<0.01) among cultivars of common beans in relation to induced procedure of determining drought tolerance as described by Michel and Kaufiman (1973) was followed. Data generated from the experiment were subjected to ANOVA using Genstat Version 14. Mean separation was done using LSD. The results revealed that gifferences exit among bean cultivars.Different concentrations of PEG created highly significant (P<0.01) different environments for common bean cultivars. Interactions of common beans and PEG concentration created highly significant different (P<0.01) environments in which seed germinated and seedlings grew. Kranskop and Small white haricots cultivars obtained highest values in five drought stress indices out of eight measured, followed by CAP 2000, Mkuzi, Nordak, RS7 and DBS 840 cultivars with highest values in four drought stress indices, lastly followed by PAN 148, PAN 9213 and DBS 310 in three drought stress indices.*

KEYWORDS: Common Bean, Cluster Analysis, Drought Tolerance, Peg.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most valued leguminous crop grown in Lesotho because of its high consumption by many house-holds, price it fetches at the formal market and its ability to fix atmospheric nitrogen for its own utilization with surplus deposited in the soil to enrich it (Morojele and Mbewe, 2014). It is grown as a sole crop or inter-cropped with maize and sorghum to increase crop intensity and mitigate risks (Kaur *et al.*1998). Common bean is produced under dryland conditions in rotation with winter wheat to increase diversity. Being a popular crop, it is grown in all agro-ecological zones of Lesotho, namely; lowlands, foot-hills, mountain and Orange river valley (Morojele and Tsikoane, 2017; Sefume, 2014). A high concentration of common bean is found in the lowlands, followed by foothills, mountains and some traces in the Orange River valley (Sefume, 2014). The production techniques used to produce this crop is of low management practices and low external inputs, characterized by poor agronomic measures, lack of managerial skills, application of inadequate fertilizer and manure, less pests control measures and untimely harvesting (Central Bank, 2015). Other natural disasters affecting yield of common beans are high level of disease and

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insect infestations, early and late frost, high and low temperature, and drought prevalence among others (Bureau of Statistics, 2014). The most serious impediment in the production of common beans is intensive drought occurrence brought by climate change (Bureau of Statistics, 2014). This problem is escalating from one year to another as mentioned by Lesotho Meteorological Services, (2015). Although it is documented, that common bean is susceptible to drought stress or water deficit, the production of this crop in many places of the world is carried out under drought stress conditions, due to insufficient water supply by rainfall and/or irrigation (Zlatev and Stoyanov, 2005). As much as 60% of bean production in the developing world occurs under conditions of significant drought stress (Leport *et al.*, 2006). This is probably the reason why the average global yield of beans remains low (<900 kg ha).

It therefore, appears that drought stress is a worldwide production constraint for common bean productivity. Some management practices can contribute to the increase of common bean yield under drought conditions, thus the identification and selection of tolerant cultivars becomes an efficient and economical production strategy. Drought tolerance implies the ability to sustain substantial yield under moderate water stress and not the ability to survive over prolonged and severe water stress periods (Ashraf, 2005). Water stress has been reported to reduce the expression of many characteristics in beans except days to flowering and moisture retention in the leaf (Farooq, 2006). In common beans, accelerated maturity of crop along with reducing grain yield and mean weight of hundred seeds following water stress have been reported (Hufsteler *et al*, 2007).

There is a wide variation among cultivars of common beans in terms of tolerance to drought. Some cultivars respond negatively to drought and give low yield while others give substantial yield. The ones that perform better and give a reasonable yield are selected, multiplied and included in the breeding programmes. Various methods have been employed to identify drought tolerant genotypes and efforts have been made in the past to screen bean varieties which differed in drought tolerance (George *et al*, 2013). Polyethylene glycol (PEG) compounds have been used to induce osmotic stress in petri dish (in vitro) for plants to maintain uniform water potential during the experimental period. PEG has been used often as water stress inducer in many studies to screen drought tolerant germplasm (Turkan *et al*, 2005). PEG is a polymer and considered as better chemical than others to induce water stress artificially (Kaur *et al*, 1998; Ashraf and Iram, 2005).

This study was conducted in Lesotho with an object of (1) verifying the differences among common bean cultivars in response to induced water deficit stress, (2) evaluating common bean cultivars against different concentration levels of Polyethylene glycol which induces drought stress in germinating seed and seedling growth and (3) identifying cultivars of common beans tolerant to drought stress and rank top performing accordingly.

MATERIALS AND METHOD

Study area

Laboratory experiment was conducted at The National University of Lesotho, Faculty of Agriculture, in the Department of Crop Science situated 34 km South East of Maseru, the capital town of Lesotho. Coordinates of The University are 29^0 26' 48 South latitude and 27^0 42' 29 East longitudes with the altitude of 1.610 m above-sea level.

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Experimental Design

Complete Randomized Design with three replications and twenty-eight (28) treatments were employed. Treatments were seeds of common bean cultivars obtained from Department of Agricultural Research in Maseru, Lesotho and concentrations of PEG-6000. The experiment was carried out from November 2016 to January 2017 to investigate the effects of PEG-induced drought on germination and seedling growth of twenty-eight common bean cultivars.

Procedure

Twenty-eight different accessions of beans were evaluated for their drought tolerance at germination and seedling stage. Water stress was simulated by non-ionic water soluble polymer polyethylene glycol of molecular weight 6000. Solution of PEG-6000 having osmotic potential of -1.0 bar as described by Michel and Kaufiman (1973) was prepared by dissolving different concentrations of PEG (117,78,39 and 0g) in 1000ml of distilled water. For control conditions, distilled water was used. Screening of these cultivars was done by allowing them to grow for 14 days under different PEG-6000 concentration. After 14 days, data were recorded for seedling traits namely; germination percentage, germination stress index, plumule length, radicle length, fresh plumule weight, dry plumule weight, fresh radicle weight and dry radicle weight under control as well as water stress conditions.

Seeds of different cultivars were surface-sterilized with 0.1% Sodium hypochlorite (w/v) for 2 minutes. Ten sterilized seeds of each cultivar were spread over a blotting paper in a petri-dish separately. Four different concentrations of PEG that were mentioned previously were added in different petri dishes every day. The whole set were placed in the growth chamber with bright diffused light, 70 - 80% relative humidity and 25 – 30 ^{0}C temperature.

Data collection

The following seed germination and seedling parameters were measured; germination rate measured at day 1 up to day 14 after planting, germination percentage at day 14, plumule length, radicle length, plumule fresh and dry weight, radicle fresh and dry weight under varying concentrations of PEG.

Data analysis

Genstat recovery Version 14 was used to analyse data collected above and generate Analysis of variance. Mean separation was done using least significant difference.

RESULTS

The analysis of variance depicted in table 1 revealed highly significant difference ($P<0.01$) among the PEG concentrations, dry bean cultivars and interaction of dry bean cultivars evaluated for germination percentage, germination seed index, plumule dry weight, plumule fresh weight, plumule length, radicle dry weight, radicle fresh weight and radicle length.

PEG Concentrations

The grand mean for germination percentage of all four different concentrations of PEG was 72.92%. The highest germination percentage was obtained where control (0 PEG) was

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employed having 95%, followed by 80% where 39g (-0.5bars) PEG was applied. The lowest germination percentage of 37.62% was exhibited in a PEG concentration of 117g (-1.5 bars). Germination seed index revealed a grand mean of 0.557 with the highest index of 1.014 where 39g PEG was added. The lowest index was found where the PEG concentration was 117g (-1.5bars). No Seed germination index (0.000) was observed for control 0.00 PEG concentration. Plumule dry weight had a grand mean of 0.01982 with the highest weight of 0.03798 obtained where 39g PEG was dissolved in the solution. The lowest plumule dry weight was 0.000g where 117g PEG was added. The grand mean of plumule fresh weight was 0.0984g with the highest weight of 0.1102g where PEG concentration was 39g and lowest weight of 0.0198g was observed with concentration of 117g PEG. The overall mean of plumule length for four PEG concentrations recorded 1.343mm with the highest and lowest being 2.182mm and 0.408mm where 10g PEG and 117g were added to the solution, respectively. Radicle dry weight had a grand mean of 0.0349g with the highest weight of 0.0558g and lowest weight of 0.0101g obtained from the PEG concentration of 39g and 117g, respectively. The overall mean of radicle fresh weight is 0.1563g with control having the highest weight of 0.3871g and the lowest of 0.0001g, respectively. The radicle length had a grand mean of 5.897cm. The longest length of 7.632cm was obtained where PEG concentration is 39g while the shortest length of 2.396cm was found where 117g PEG was applied. Means are depicted in table 2 below.

Variability in dry bean cultivars

Among the twenty-eight cultivars of dry beans used in this study, highly significant differences ($P<0.01$) were obtained on the above-mentioned parameters. Mkuzi, Small white haricots and Nodak obtained the highest average germination percentage. The lowest average germination percentage was experienced by DBS 360, PAN 9216 and PAN 9292. Germination seed index revealed that SW1, Mkuzi and Small white haricot scored highest values. DBS360 got very low value, followed by Seederburg. The cultivars with high plumule dry weight were DBS 310, DBS 360 and DBS 830 and the lowest dry weight and PAN123 and RS 5. The cultivars with high plumule fresh weight were CAP2000 and DBS360 while KRANSKOP HR1 and Mkuzi had the lowest plumule fresh weight. Plumule length was found longest in TEEBUS RR1, Small white haricot and Mkuzi while the shortest length was obtained in Kranskop HR1, RS 5 and Kranskop. High radicle dry weight was observed in CAP2000 and DBS 310 and the lowest radicle dry weight was obtained in PAN123 and PAN9216. Radicle fresh weight was high in CAP2000 and DBS310 and low in Ruby, DBS360 and SE1. The longest radicle length was observed from Teebus, Mkuzi and NAU 45. Means are depicted in table 2 below.

Interaction of PEG concentrations and dry bean cultivars

The interaction of cultivar with PEG showed highly significance ($P<0.01$) for germination, plumule dry weight, plumule flesh weight, radicle fresh weight and radicle length. Significant difference ($P<0.05$) was obtained in plumule length and radicle dry weight. Germination stress index was found to be non-significant. Interaction of dry bean cultivars and PEG concentration at 117g (-1.5bars) revealed low germination of 10% and PEG concentration of 39g showed a germination of 96%. Control where PEG was not applied germination percentage was 100% with most of the cultivars. While the lowest germination under control was 83%. Regarding germination seed index, no significant difference was found among 28 cultivars of dry beans. Highly significant difference ($P <0.01$) was obtained among cultivars for plumule dry weight. Where 117g of PEG concentration was applied, plumule length was 0 and could not be measured by microns, thus negligible. The longest dry plumule length was 0.07667mm where 39g (-0.1bars) PEG was applied. Control had the longest dry plumule length in all cultivars.

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Plumule fresh weight exhibited a significant difference (P<0.05) among dry bean cultivars. PEG concentration of 117g resulted in a germination of mostly 0 in all cultivars. Most of the cultivars showed a higher dry weight under control, followed by PEG concentration of 39g. Radicle dry weight revealed significant difference among cultivar x PEG concentrations. The highest weight obtained was 0.1100g while the lowest was 0 where 39g and 117g were applied respectively. The interaction of dry bean cultivar and PGE concentration was highly significant (P<0.01). The lowest radicle fresh weight was 0 where 117g was applied while the highest was 0.2267g under PEG concentration of 39g. Control recorded the highest radicle fresh weight of 0.6533g and lowest radicle fresh weight was 0.11g. Interaction of radicle length and PEG concentration was highly significant (P<0.01). The longest length was 11.133cm found a concentration of 39g while the shortest was 0.133cm found in a concentration of 117g PEG concentration.

Based on the interaction of common bean cultivars and different concentration of PGE, cultivars which performed well in the concentration of 78g (standard in most research work done) were considered to be tolerant to induced drought stress. No cultivar of common bean obtained a high value in all the drought tolerance indices. However, Kranskop and Small white haricots cultivars obtained highest values in five drought stress indices out of eight measured, followed by CAP 2000, Mkuzi, Nordak, RS7 and DBS 840 cultivars with highest values in four drought stress indices, then lastly followed by PAN 148, PAN 9213 and DBS 310.

Table 1. Analysis of variance for seed germination parameters

		Mean Square							
SOURCE OF VARIATION	**df**	**Germination percentage**	**Germination seed Index**	**Radicle length**	**Plumule length**	**Radicle fresh weight**	**Plumule fresh weight**	**Radicle dry weight**	**Plumule dry weight**
Varieties	27	1659.0**	0.8544**	29.096**	0.49533**	0.02782**	0.011916**	0.0019604**	0.0009126**
PEG Conc	3	51944.0**	19.4079**	487.217**	147.39004**	2.30611**	0.835161**	0.0328393**	0.0291020**
Varieties XPEG Conc	81	573.4**	0.4804	5.699**	0.13401	0.01648	0.009881**	0.0010734	0.0003577**
Error	226	192.3	0.3692	2.740	0.09610	0.01078	0.005121	0.0007688	0.0001690
Grand mean		72.92	0.557	5.897	1.343	0.1563	0.0984	0.0349	0.01982
LSD		22.310	0.9777	2.6633	0.4988	0.16704	0.11514	0.04461	0.020920

** Highly significant (P< 0.01)
* Significant (P<0.05)

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TABLE 2 Means for PEG Concentration

PEG CONC.(g)	GM%	GSI	RADICLE LENGTH	PLUMULE LENGTH	RADICLE FRESH WEIGHT	PLUMULE FRESH WEIGHT	RADICLE DRY WEIGHT	PLUMULE DRY WEIGHT
0	95.95	0.000	7.317	2.182	0.3871	0.2349	0.0294	0.03321
39	80.36	1.014	7.632	1.626	0.1496	0.1102	0.0558	0.03798
78	77.74	0.898	6.244	1.157	0.0882	0.0286	0.0444	0.00810
117	37.64	0.317	2.396	0.408	0.0001	0.0198	0.0101	0.00000

TABLE 3. Means for varieties

VARIETIES	GM%	GSI	RADICLE LENGTH	PLUMULE LENGTH	RADICLE FRESH WEIGHT	PLUMULE FRESH WEIGHT	RADICLE DRY WEIGHT	PLUMULE DRY WEIGHT
CAP200	80.83	0.650	6.683	1.625	0.2533	0.1708	0.0642	0.03167
DBS 310	68.33	0.625	3.925	1.433	0.2250	0.1075	0.0508	0.04167
DBS 360	53.33	0.292	4.000	1.417	0.0867	0.1633	0.0267	0.03833
DBS830	75.00	0.483	6.858	1.467	0.2542	0.1292	0.0450	0.03333
DBS 840	83.33	0.567	7.575	1.383	0.2000	0.1000	0.0417	0.01917
KAMIESBURG	69.17	0.383	5.575	1.192	0.1725	0.0775	0.0483	0.01333
KRANSKOP	64.17	0.383	4.025	1.092	0.1675	0.0667	0.0617	0.01583
KRANSKOP HR1	64.17	0.375	4.908	1.058	0.1150	0.0550	0.0300	0.01083
MKUZI	96.67	0.875	8.208	1.550	0.1383	0.1375	0.0258	0.02333
NODAK	95.83	0.783	5.967	1.267	0.1450	0.0967	0.0392	0.01333
NUA 45	88.33	0.567	7.750	1.267	0.1542	0.0683	0.0433	0.02167
OPS RS 4	75.00	0.450	5.808	1.267	0.0833	0.0717	0.0342	0.01500
PAN 123	64.17	0.533	6.100	1.558	0.1133	0.0867	0.0150	0.00917
PAN148	70.00	0.508	5.567	1.408	0.1525	0.1133	0.0333	0.02667
PAN 9213	77.50	0.642	5.650	1.100	0.1858	0.0633	0.0425	0.01417
PAN 9216	58.33	0.400	4.967	1.242	0.1467	0.1075	0.0167	0.01333
PAN 92922	60.83	0.358	4.208	1.200	0.1700	0.0917	0.0317	0.01333
RS 5	62.50	0.417	5.033	1.067	0.0892	0.0592	0.0175	0.00916
RS 6	59.17	0.358	4.808	1.308	0.1467	0.1008	0.0208	0.02250
RS 7	74.17	0.483	5.867	1.475	0.1642	0.1133	0.0342	0.02833
RUBY	72.50	0.592	4.842	1.192	0.0692	0.0992	0.0225	0.01583
SEEDERBURG	60.83	0.300	4.367	1.125	0.1858	0.0883	0.0325	0.01667
SMALL WHITE	96.67	0.875	9.825	1.767	0.1442	0.0883	0.0242	0.01167
SUG 131	68.33	0.467	6.133	1.325	0.1833	0.1408	0.0492	0.02500
SW 1	85.00	1.667	7.458	1.550	0.0992	0.0708	0.0233	0.01917
TEEBUS RR1	75.00	0.583	9.208	1.817	0.1775	0.1450	0.0392	0.02500
TYGERSBURG	70.00	0.392	5.092	1.225	0.2133	0.0692	0.0375	0.01333

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DISCUSSION

The general trend in the results of this study showed germination percentage, germination stress index, plumule dry weight, plumule fresh weight, plumule length, radicle dry weight, radicle fresh weight and radical length increased with decrease in the concentration of PEG. Where no PEG was applied (distilled water used), all the parameters were higher than where there was PEG at any concentration.

PEG Concentrations

Polyethylene glycol (PEG) possessed properties that reduce the available water necessary for seed germination and seed seedling growth. The reason being that PEG had higher osmotic potential of absorbing water that was otherwise required by germinating seed or growing seedling. As the concentration of this chemical was increased from lower concentration to the higher concentration, the water available to seed germination became scarce. This was a situation that occurred when the seed was planted in a dry soil during drought conditions. PEG was used by many researchers in inducing drought in seeds and seedling to simulate conditions that exist in the field under dry soil conditions or drought (Heikal and Shaddad, 1982; Kuhad *et al*., 1987). Turhum (1997) emphasised that PEG caused osmotic stress and could be used as a drought inducer. Similar results were obtained by Smok *et al*. (1993) and Hu and Jones (2004) who used PEG to induced osmotic stress on germinating seed and seedlings of cotton, peas, wheat, beans and sorghum. Seed germination is one of the most critical and sensitive stage in the life-cycle of the plant (Ashraf and Mehmood, 1990). When the germinating seed is exposed to water deficit conditions, it compromised the seedling establishment (Albuquerque and Carvalho, 2003). Germination percentage and germination stress index declined as PEG concentration increased. This showed that less free available water became unavailable to the seed. PEG potential exerted more influence on seed germination rate and percentage (Bewley and Black, 1994).

Seedling growth parameters measured such as fresh weight, dry weight and length of plumule and radicle also decreased as the concentration PEG increased. This implied that the water available to the seedlings became scarce as the PEG concentration increased. PEG absorbed more water since it had high osmotic potential. Osmotic stress delayed the emergence of both radicle and further development of seedling. These results were comparable with the findings of Turhum (1997). They indicated that decline in the seedling development was due to osmotic potential of PEG or ionic effects or a combination of both. In this study, there was a decreasing pattern in the water uptake by the seeds in comparison with control among twenty-eight bean cultivars. This could be associated with external water potential which declined because of diffusivity of seed coat to water at low water potential. PEG reduced osmotic potential of the external medium and decreases water availability for germinating seed.

Variability in dry bean cultivars

Great variability among twenty-eight cultivars of beans was observed with some, having high values while other showed low values for the parameters measured. The variability was attributed to different genetic make-up of cultivars being under study. Cultivars such as Mkuzi, small white haricot and Nordak were scored very high in most of the parameters while the cultivars with the lowest scores DBS 360, PAN 9216 and PAN 9292. The other varieties felt within the range. The variation suggested that, a choice can be made among the cultivars for water deficit tolerance. Dutta and Bera (2008) screened fifteen mung-bean genotypes for

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drought tolerance using different concentrations of PEG and found three cultivars of mung-bean being tolerant while another four were susceptible to drought induced conditions. Similarly, Giancarla *et al*. (2012) evaluated barley cultivars using laboratory experiment of PEG and obtained a great variation among cultivars ranging from high scores to low scores. Nonetheless, this study also revealed that some cultivars responded the same to PEG when particular concentration was used. This implied that the cultivars were sharing similar genes of drought stress. The common bean cultivars that share similar values were Kranskop and Kranskop HRI, VERNA and RABY and many others.

Interaction of PEG concentrations and dry bean cultivars

The analysis of variance performed revealed a great variation in the interaction of PEG concentration and dry bean cultivars. Variation in the interaction was observed between cultivars and PEG concentrations. Each cultivar reacted differently under each PEG concentration implying their genetic make-up. Nonetheless, there were some cultivars that reacted same, showing similarities in their gene composition regarding tolerance to stress. Physiology of the genes indicates that the genes produce proteins which in turn produce enzymes. Enzymes are responsible for reactions such as adaptions to water stress or chemical induced stress. Genes responsible for adaption may be high or low in response to water stress and each cultivar possess either of the two but not both. All the parameters under study exhibited highly significant difference in the interaction of PEG concentration and bean cultivars except for radicle fresh weight and plumule length. Different concentration of PEG simulated different types of climate conditions where a locality may be favourable for some cultivar and unfavourable for others. In this study, control was considered the most conducive locality whereas increasing PEG concentration was synonymous with increasing severity of the unfavourable conditions. Khakwani *et al*. (2011) conducted a similar study by screening eight cultivars of wheat and observed a wide variation among the interactions of PEG concentration and wheat cultivars. They further established that, the PEG concentration of 78g (-1.0 bars) produce best results for this type. Similarly, Giancaria *et al*. (2012) found the interaction of different barley cultivars and different PEG concentrations resulting in a great variation in the parameters that they were studying.

The study confirmed that the cultivars of common beans were significantly different from each other in respond to induced water deficit stress (Table 1). It implied that the genetic make-up of the cultivars were different. Moreover, different concentrations of PEG created different environments for germinating seeds and growing of seedlings hence, a decrease in the values of induced water deficit stress parameters as concentration of PEG increased. Where control (distilled water) was applied, all the indicators of the parameters measured were highest. Furthermore, interaction of PEG and cultivars of common beans were also highly significant resulting in some cultivars out-performing the others under high concentration of PEG. It was in this interaction where the cultivars of common beans with tolerance to induced water deficit stress were identified. And these cultivars were ranked accordingly.

CONCLUSION

Cultivars of common beans showed a highly significant difference in relation to induce water deficit stress. Different concentrations of PEG created highly significant different environments for common bean cultivars. Interactions of common beans and PEG

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concentration also created highly significant different environment in which seed germinated and seedlings grew. Kranskop and Small white haricots cultivars obtained highest values in five drought stress indices out of eight measured, followed by CAP 2000, Mkuzi, Nodak, RS7 and DBS 840 cultivars with highest values in four drought stress indices, then lastly followed by PAN 148, PAN 9213 and DBS 310.

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