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Full Length Research Paper

Mitotic chromosome studies of some accessions of African yam bean *Sphenostylis stenocarpa* (Hochst. Ex. A. Rich.) Harm.

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In this study the chromosome number, chromosome length and mitotic indices of 21 accessions of African Yam Bean (AYB) were determined. To accomplish this, the best time to harvest growing root tips was identified by measuring mitotic index of chromosomes prepared from root tips of accession TSs148 collected at two hours interval within a 24 h study period from 8 am to 6 am of following day. Cell division occurred throughout the day and at night with the rate of division increasing to a peak at midday. The highest percentage of metaphase cells (34.01%) was obtained from roots harvested at about 12:00 noon. In terms of the root tip to shoot tip that gave a higher mitotic index a ratio of 13:8 was observed among 21 accessions studied. A diploid chromosome count ranging between 2n = 20 to 24 was observed with the chromosome number 2n = 22 being the most frequent. The chromosomes of the accessions studied were generally small in size ranging between 0.58 to 1.84 µm. On the basis of chromosome size, the different accessions of AYB could be divided into three different groups. This information, if utilized, will pave the way for further progress in karyological and cytogenetic research towards improvement of this plant.

Key words: *Sphenostylis stenocarpa,* chromosome number and length, mitotic index, genetic variation.

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa* [Hochst. Ex. A. Rich.] Harm) like other tuberous legumes (for example, *Pachyrhizus* sp.) belongs to the Fabaceae family and the order Fabales. It is the most important cultivated species in the genus *Sphenostylis* (Potter and Doyle, 1992; Okigbo, 1973) and by its name is indigenous to tropical Africa. It was believed that the AYB originated from Ethiopia and had spread to most parts of Africa. It is common in Central and West Africa, particularly in Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo (Porter 1992). It was introduced into Ghana from Togo in 1958 (Adansi, 1975). It is found in forests, open and wooded grasslands, rocky fields as well as marshy grounds, occurring both as a weed and a cultivated crop (Duke et al., 1977; Potter, 1992). It grows on a wide range of soils including acid and highly leached sandy soils at altitudes, ranging from sea level to 1950 m (Duke et al., 1977). It forms slightly woody pods which are linear and measure up to 30 cm containing 20 to 30 seeds which mature within 170 days. The seeds are very hard and diverse in colour and shape usually spherical, ellipsoid with dark-brown, creamy-white or brownish yellow colour (Duke et al., 1977; Edem et al., 1990).

This indigenous crop has potential for food security in Africa. The seeds and tubers are the two major organs of economic importance as food for Africans. However, there are cultural and regional preferences. In West Africa, the seeds are preferred to the tubers and the tubers are relished in East and Central Africa (Potter 1992). The crop replaces cowpea in some parts of southwestern Nigeria (Okpara and Omaliko, 1995). AYB seed is well balanced in essential amino acids and has a higher amino acid content than that found in pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001). The whole seeds were found to be rich in

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potassium (649.49 mg/100 g) and phosphorus (241.21 mg/100 g) (Oshodi et al., 1995). Seeds are also rich in magnesium, calcium, iron, and zinc but low in sodium and copper (Edem et al., 1990). Achinewhu et al. (2003) analyzed raw samples of AYB and cowpea processed into akara, moimoi and porridges for chemical, functional and sensory properties. Sensory evaluations showed that AYB do not differ significantly from cowpea. The crop also nodulates profusely and probably has high nitrogen-fixing ability, thereby helping to replenish soil nitrogen (Klu et al., 2001). AYB seeds contain lectins, a group of carbohydrate binding proteins, found to have strong insecticidal effect against cowpea weevil, *Callosobruchus maculatus* (Machuka et al., 2000).

Although the vast genetic and economic potentials of AYB have begun to be recognized, especially in reducing malnutrition among Africans, the crop has not received adequate research attention compared to major legumes such as cowpea, groundnut and soybean. It is thus a minor legume classified as neglected, underutilized or underexploited species (Saka et al., 2004). Its usefulness and attractiveness to consumers could be enhanced if it receives adequate attention and breeding efforts are geared towards its improvement. AYB needs to be genetically improved for farmer preferred traits like disease resistance, drought tolerance and early flowering to reduce the length of the crop cycle; as well as consumer preferred traits like reduced seed coat hardness or increased permeability to make for easier cooking and increased nutritional value and/ or reduced antinutritional factors which are reported to cause discomfort and flatulence when consumed (Onyeike and Omubo–Dede, 2002).

Genetic biodiversity especially within species provides the resources for plant breeding and improvement programmes. Essential germplasm of cultivated food crop species in tropical Africa, particularly those not receiving research attention are in danger of getting lost (Devos et al., 1980). It is therefore important to conserve and characterize AYB germplasm and evaluate its genetic potential for useful traits. Information provided by characterization can be used in identifying and also developing new and promising AYB genotypes, which could be recommended directly to the farmers and other end users.

Few reports are available on its diversity with respect to presence of nutritive and anti-nutritive factors (Ajibade et al., 2005; Betsche et al., 2005), protein content (Uguru and Madukaife, 2001), seed colour and colour pattern (Oshodi et al., 1995), seed shape, seed size and other vegetative and reproductive morphological traits (Adewale and Dumet, 2009; Adewale et al., 2010). However there is no report on cytological or karyological studies in this species. Since the chromosome is directly responsible for inheritance of traits, the study of AYB chromosome parameters is pertinent to its genetic improvement.

Some of the very important and basic measures of inter- or intra-specific variability with respect to cytogenetics of any crop include chromosome number, chromosome size and morphology, and mitotic index. In order to determine these parameters a reliable procedure for somatic chromosome preparation must be established. However, no successful methodology for visualization of AYB chromosomes has been reported yet, probably due to its small size. Chromosomes are best studied at metaphase and the number of cells in metaphase as well as mitotic index could vary with the time of the day when meristematic cells are collected for chromosome preparation (Osuji and Owei, 2010). Moreover, some reports have shown that root tips do not give good results for mitotic chromosome studies (Uguru et al., 2005). Therefore, the aims of this study were to determine the best time of day for collection of root tips for AYB metaphase chromosome evaluation, to compare mitotic indices of root tip against shoot tip chromosome preparations, and to determine variations in chromosome number and chromosome length and mitotic index among twenty one accessions.

MATERIALS AND METHOD

Twenty one accessions of African Yam Bean used in this study were obtained from the gene bank of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. All accessions have their origin in Nigeria except TSs 77 which was collected from Ghana. This work was carried out in two phases. The first phase involved the determination of the best time at which the germinating root can be harvested to obtain the highest percentage of metaphase cells using accession TSs148, and consequently, obtain the best chromosome count. Seeds were germinated in the laboratory on moistened filter paper or in petri dishes at room temperature for seven days. Fresh roots of the germinated seeds were harvested at two-hourly intervals according to Osuji and Owei (2010), starting from 8:00 am to 6:00 am, the following day. For the second phase, the sprouting roots and shoots of the germinated seeds of the 21 accessions of AYB were harvested at noon as determined from the first study to be the hour with the highest metaphase cells. The roots and shoots tips of about 1 to 2 cm long were harvested, and pre-treated in 0.2% colchicine solution for 1 h. The pre-treated roots and shoots were rinsed thoroughly with water and fixed in a freshly prepared Carnoy's solution (3: l ethanol acetic acid) for 24 h at room temperature. The tips were rinsed in water and hydroysed at 65ºC for 5 min in a 1M HCl solution. About 1mm of root (or shoot) tips were then teased into the slide, macerated with needles and stained with a drop of FLP Orcein (Olorode, 1974, Osuji, 2003). They were covered with a slip and cells were squashed with the thumb and the stained material was allowed to stand for 35 to 40 min to allow the chromosomes absorb enough stain. A firm pressure was applied with the thumb and gentle tapping was made on the cover slip with the base of a ballpoint pen to help flatten the cells. Excess stain was wiped off and permanent slides prepared. Cells with well spread metaphase chromosomes were used for the karyotypic studies. Photomicrographs were taken using OLYMPUS research microscope with a digital camera attached.

Mitotic index was determined by counting and differentiating the cells on the slides obtained from the first study under the microscope at x100 magnification using immersion oil. The

numbers of dividing cells at the different stages of mitosis - prophase, metaphase, anaphase, telophase and interphase- were obtained for each 500 cells counted in a slide representing the various root tip collections (in the day and at night) Table 2. This was used to calculate the percentage of cells at a particular mitotic phase in each two- hour interval. It was calculated as number of cells in mitosis divided by total number of cells counted.

From the values obtained on the Mitotic Index the time to obtain the best chromosome count was determined. The mitotic index was then calculated for the root and shoot tip of each of the 21 different accessions of AYB. The chromosomes were counted with the aid of a microscope at x100 magnification objective under oil immersion in at least three cells at the metaphase stage in each slide for the 21 accessions of AYB and the chromosome number with the highest frequency was chosen.

Chromosome length was determined using ocular micrometer, stage micrometer, light microscope (at x100 objective). The calibration constant was first determined for the light microscope. Each slide was placed on the stage to measure the longest and shortest chromosomes in at least 10 cells for each accession using the ocular micrometer scale. The average length of chromosome for each accession was determined.

RESULTS

In the first phase of this study, cell division occurred at all times round a 24 h clock (Figure 1). The rate of cell division was found to vary at different hours of the day and the variation between the night and the day was found to be large. The percentage of dividing cells was higher in the day-time (8:00 am to 8:00 pm) than at night. Between 6:00 and 8:00 am the number of cells involved in mitotic division radically started to increase. The highest number of cells at the prophase and interphase stages was observed at about 6:00 am. The highest number of cells at the metaphase stage was observed between 10:00 am and 12:00 noon and the highest number of dividing cells was observed between 12:00 noon and 2:00 pm, which is equivalent to the time at which the lowest percentage of cells at interphase was observed.

A chromosome number of 2n = 20 to 24 was recorded across all 21 accessions of AYB. Ten accessions had a chromosome number of 2n = 22, 9 accessions had chromosome number of 2n = 20 whereas only two accessions, TSs 41 and TSs140, had a chromosome number of 2n = 24. No case of polyploidy was observed. Overlapping of some chromosomes in the genome was also observed (Table 1).

The chromosome length varied from 0.58 to 1.84 µm across the 21 accessions studied. On the basis of the sizes of the mitotic chromosomes obtained from the different accessions of AYB, they could be divided to three different groups. Accessions belonging to the first group had their chromosome size less than or approximately equal to 1 µm and TSs77 belongs to this group. The second group contained accessions whose chromosome sizes are larger than or are approximately equal to 1 µm and this include TSs5, TSs14, TSs17, TSs34, TSs41, TSs42, TSs51, TSs113, TSs138, TSs140, TSs155 and TSs157. Accessions belonging to the third group had a chromosome size ranging from that of the first group to that of the second group and this include TSs19, TSs120, TSs123, TSs139, TSs148, TSs150, TSs152 and TSs156 (Table 1).

Mitotic indices from 41.4 to 86.7% for the shoot tip and 42.4 to 88.1%, for the root tip, were observed across the 21 accessions studied. In 13 accessions, TSs5, TSs14, TSs19, TSs34, TSs41, TSs120, TSs123, TSs140, TSs148, TSs152, TSs155, TSs156 and TSs157, the root tips showed a higher mitotic index than shoot tips while the reverse was the case for the remaining eight accessions. TSs41 had the highest mitotic index for both root and shoot tip (Table 1). Chromosome visibility appeared to be equally good from both, root and shoot tip preparations (Figures 2 and 3).

DISCUSSION

The observation of cell division at all times round the day is an indication of the fact that meristematic cells divide continuously. However, at night, most of the meristematic cells rested and only a few cells continued to divide, as is indicated by the reduction in the percentage of cells undergoing division. Hence, there were always cells at the various stages of the mitotic division at all times of the day, although the rate may be varied at different times of the day. The observation of the highest number of metaphase cells at about 12:00 noon corresponds to the period when most plants record a very high photosynthetic level due to maximum availability of sunlight and thus a very high rate of synthesis of metabolites and energy, in the form of ATP. The production of ATP is of great importance during cell division as it is needed to synthesize the different enzymes and provide energy needed for cell division (Klug et al., 2006). It was noted that there was a slight difference between the peaks of metaphase of the African Yam Bean accession, TSs148, and those reported for one of the accessions of *Colocasia* by Ekanem and Osuji (2006) and *Treculia africana* by Osuji and Owei (2010). This implies that the peaks of photosynthesis as well as mitosis may vary between species and possibly within species, as the accessions of *Colocasia* studied by Ekanem and Osuji (2006) did not all have the same metaphase peak, and this fact could be exploited taxonomically.

The observation of variable chromosome number, 2n = 20 to 24, within the species suggests the occurrence of aneuploid series as was also observed in *Citrullus lanatus* by Idehen et al. (2006). This variation in the chromosome across the accessions studied might be as a result of natural effect (suggesting that the species is still undergoing some evolutionary process with respect to chromosome number) or as a result of artificial effect (induced by the drug, colchicine used in the pretreatment).

Table 1. Chromosome number, chromosome length and mitotic indices for the root and shoot tips of 21 accessions of African Yam Bean.

Accession number	Chromosome number (2n)	Chromosome length (µm)	Mitotic Index (%)	
			Shoot tip	Root tip
TSs 5	22	1.00-1.53	68.6	76.5
TSs14	22	1.00-1.32	55.2	69.8
TSs17	20	1.00-1.44	67.4	66.7
TSs19	22	0.79-1.11	66.2	84.4
TSs34	22	0.95-1.84	64.4	75.5
TSs41	24	0.95-1.26	86.7	88.1
TSs42	20	1.00-1.48	75.4	51.4
TSs51	20	1.00-1.32	62.7	58.6
TSs77	20	0.58-1.05	59.1	57.9
TSs113	22	1.05-1.68	73.0	68.8
TSs120	22	0.88-1.48	47.1	47.6
TSs123	22	0.86-1.44	64.0	65.0
TSs138	20	1.05-1.15	65.1	51.4
TSs139	20	0.74-1.16	78.0	42.4
TSs140	24	0.95-1.26	61.8	70.0
TSs148	20	0.79-1.21	65.5	67.4
TSs150	20	0.86-1.34	60.5	58.7
TSs152	22	0.89-1.26	50.5	62.4
TSs155	20	0.95-1.11	55.6	60.8
TSs156	22	0.95-1.05	52.6	57.4
TSs157	22	0.96-1.58	41.4	64.5

Table 2. Mitotic cell division in two hours interval.

Hour	Prophase	Metaphase	Anaphase	Telophase	Interphase
8:00	18.63	26.67	35.12	11.5	12.08
10:00	10.03	27.96	44.07	11.55	6.38
12:00	15.87	34.01	32.85	12.97	4.32
14:00	6.95	31.72	45.62	12.39	3.32
16:00	13.41	29.63	32.94	13.88	10.12
18:00	12.21	25.19	43.89	13.36	5.34
20:00	10.04	18.41	48.54	16.32	6.69
22:00	17.19	22.5	42.81	7.81	9.69
00:00	10.75	21.51	46.24	11.83	9.68
2:00	13.27	23.91	38.94	11.5	13.27
4:00	18.25	20.31	37.88	11.47	13.54
6:00	29.54	18.1	23.92	11.38	17.07

The time is in 24 h mode and the data collected is in percentage of dividing cells at each stage of cell division during each time interval.

This variation could also be as a result of interbreeding of different populations within the species. This could explain the variation of the chromosome counts of some of the accessions showing aneuploidy changes from 2n = 22 (chromosome count with the highest percentage). From this, the variation was possibly by the loss of one pair of chromosomes called nullisomy as in 2n = 20, or by the gain of a pair of chromosomes called tetrasomy as in 2n = 24. Variation in the sizes of the chromosomes of the different accessions may be due to the different environmental factors (Stace, 2000), resulting in the deletion or addition of a segment

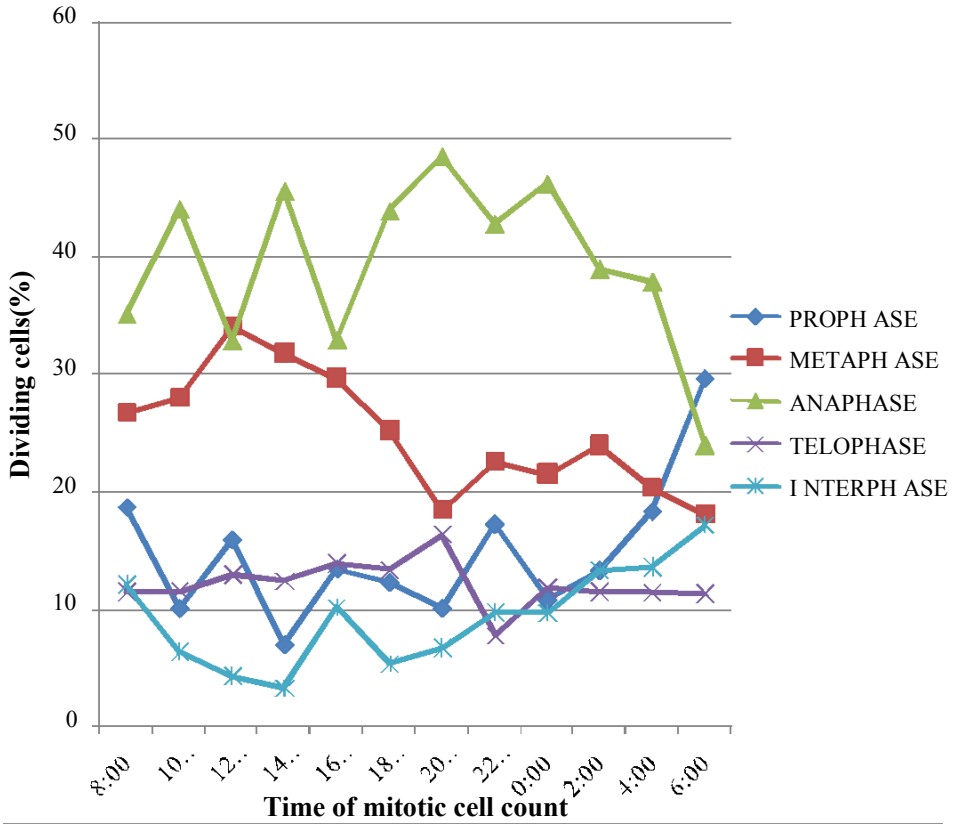

Figure 1. Percentage of dividing cells of the root tips of TSs148 at the various stages of division within a 24 h cycle.

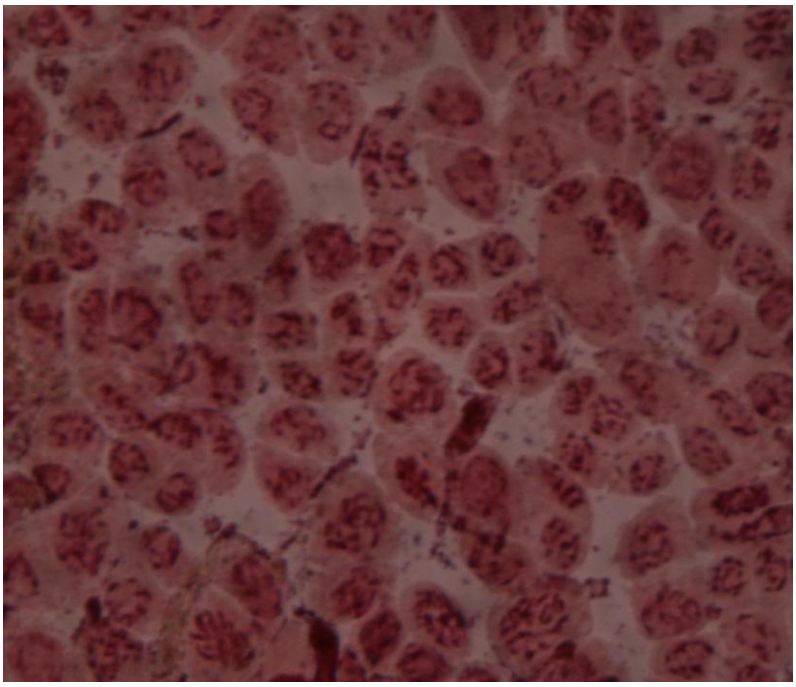

Figure 2. Chromosome of the shoot tip of African Yam Bean TSs41 (Mag. X100).

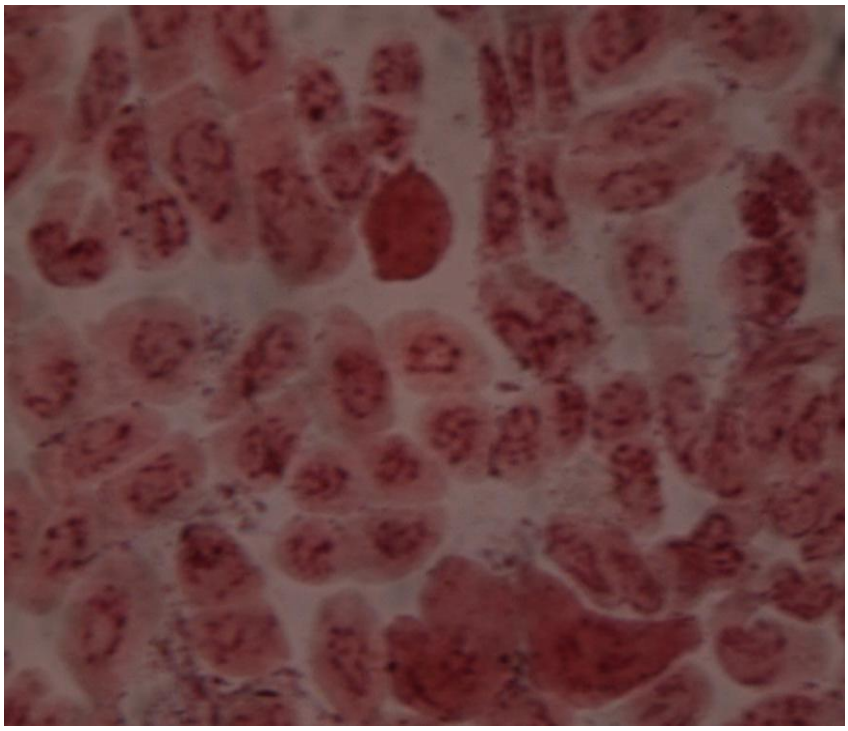

Figure 3. Chromosome of the root tip of African Yam Bean TSs41 (Mag. ×100).

of chromosome. TSs 77 which belong to a distinct group based on chromosome size is the only accession from Ghana. This suggests that geographical origin has a strong influence on chromosome length. The remaining two groupings based on chromosome size could imply that higher genetic similarity occurs within these groups and therefore to achieve higher genetic diversity parents from these two groups could be crossed. Chromosome size variation could also be as a result of secondary factors such as the degree of condensation of chromosome length, the degree of pretreatment and the degree of stain uptake by the chromosomes.

Accessions with high mitotic index is an indication of a high rate of proliferation of cells at a particular time and this indicate a better adaptability to an environmental variable at a particular time than an accession with a low mitotic index in the same environment. The observation of the ratio of 13:8 among the 21 accessions, in terms of the root tip to shoot tip that gave a better mitotic index, however, this is not enough to assume that the root tip is best suited for mitotic study in AYB. Thus at this stage of work, the shoot tip is equally as good as the root tip in mitotic studies in AYB. It should be noted that although the meristematic tips of all the accessions worked with were harvested just before the hour of 12:00 noon based on the work with TSs148 whose mitotic peak was found to be at that hour, it does not necessarily mean that the peak of mitosis for all the accessions of AYB is at 12.00 noon. This is because there might be slight differences in the mitosis peak of different accessions of a particular species as was reported for *Xanthosoma* and *Colocasia* by Ekanem and Osuji (2006) in which most cultivars had metaphase peak between 12:00 noon and 2:00 pm except for a single cultivar of *Colocasia esculenta*, NCY 00Sa, which had a metaphase peak between 2:00 pm and 4:00 pm.

Conclusion

The variation in the chromosome number, chromosome length and mitotic index of the root and shoot tips observed in the different accessions used in this study is an indication of the great genetic diversity of this species. This information will pave the way for further progress in cytological and cytogenetic research towards the improvement of this crop plant.

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