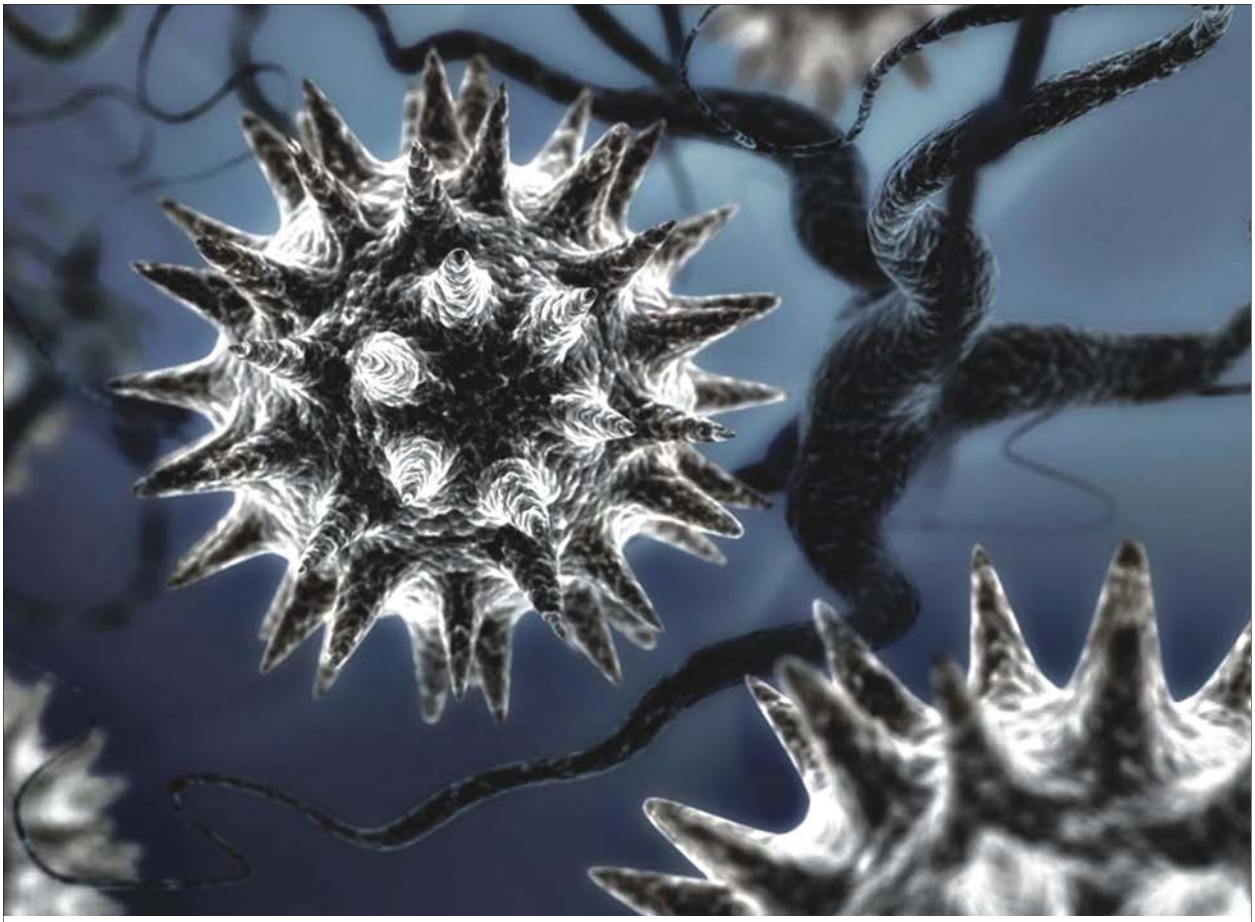

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Reaction of *Amaranthus hybridus* L. (Green) to *Telfairia Mosaic Virus* (TeMV) Infection

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ABSTRACT

The reaction of *Amaranthus hybridus* to *Telfairia mosaic virus* (TeMV) infection was investigated. Infected and healthy (control) leaf samples were obtained, pulverized and analyzed to determine the nutritional quality of the vegetable due to TeMV infection. Results obtained revealed that the virus caused significant ($p<0.05$) reductions in fibre (50.9%), fat (49.7%) and protein (32.5%) in infected samples compared to healthy ones. The virus also engendered significant reductions in the contents of Na, P, Fe, Mg, Cu and Ca with marginal reduction for K in infected samples when compared to the healthy. The TeMV caused significant reduction in phytic acid while infection led to increases in total oxalate, soluble oxalate and hydrocyanide acid. The virus caused significant reductions ($p<0.05$) in vitamin A (55.43%), C (43.1%), B_1 (34.6%), B_2 (26.6%), B_6 (17.8%) and B_3 (13.1%), respectively while, reduction in vitamin E was insignificant. The reaction of amino acids profile of *Amaranthus hybridus* to TeMV infection revealed significance decrease in methionine (35.3%), valine (34.1%), cysteine (31.9%), arginine (26.1%), isoleucine (25.9%), glycine (22.8%), lysine (17.3%), threonine (15.0%), phenylalanine (12.1%) and leucine (11.8%). Increases in infected samples were obtained for glutamic acid (19.8%), aspartic acid (9.5%) and proline (8.9%). *Amaranthus hybridus* reaction to TeMV infection revealed marked reductions in the nutritional quality of the vegetable that is a major source of nutrient for both rural and urban dwellers.

Key words: *Amaranthus hybridus, Telfairia mosaic virus*, nutritional quality

INTRODUCTION

Amaranthus hybridus popularly (called smooth Amaranthus or amaranth or pig weed) is cultivated in several areas of the world including south America, Africa, India, China and the United States (He and Corke, 2010). *Amaranthus hybridus* is an annual herbaceous plant. It is a common species in waste places, cultivated fields and barnyard. The commercial production of *Amaranthus hybridus* is increasing throughout the world as an important alternative food source (Kauffmann and Weber, 1990; Rawate, 1983). *Amaranthus hybridus* is cultivated on a commercial scale in southern Nigeria. It constitutes a major part of the diet of the people in middle and Southern parts of Nigeria, where they are mostly used in soups preparation (Oke, 1983; Mepba *et al*., 2007). In Congo, their leaves are eaten as spinach or green vegetables (Dhellot *et al*., 2006). In Mozambique and West Africa the leaves are boiled and mixed with a groundnut sauce are eaten as salad (Oliveira and de Carvalho, 1975; Martin and Telek, 1979). This vegetable has also been reported to contain squalene which has beneficial effects on cancers and industry (Rao *et al*., 1998; Smith, 2000; He *et al*., 2003).

Developing countries of the world depend on starch-based foods as the main staple food for both energy and protein supply. Protein deficiency which is prevalent among the poor populace as documented by FAO is accounted for in part. In Nigeria, where the daily diet of people is dominated by highly starchy foods. Vegetables serve as indispensable constituent in the diet (Akubugwo *et al*., 2007).

The production of *A. hybridus*, a highly nutritious leafy vegetables of tropical West Africa is reported to be limited by a number of diseases such as wet rot of leaves and young stalks caused by *Choanephora cucurbitarum* , damaging of the flowering head caused by lygus bug *Lygus lineolarius* and curly top diseases most of which are of viral etiology (Maboko, 1998). Worldwide, diseases caused by virus have been recognized to constitute one of the major factors limiting vegetable production (Grogan, 1980). *Amaranthus Mosaic Virus* (AMV) was also reported on Amaranthus in Nigeria (Thottappily, 1988). *Amaranthus* species serve as experimental host to a number of viruses (Nyamupingidza and Machakaire, 2003). *Amaranthus* species have been reported as susceptible host of TeMV (Shoyinka *et al*., 1987). The virus was reported to be seed-borne in *Telfairia occidentalis* (Anno-Nyako, 1988). Infected leaves exhibited mosaic severe leaf malformation and distortion and reduced leaf size.

Studies by Akubugwo *et al*. (2007) and Maiyo *et al.* (2010) centered on phytochemical, mineral and antimicrobial activity of leaves of *A. hybridus*. Reports also exist on the performance of *A. hybridus* as affected by enrichment of compost with urea during composting (John and Udoinyang, 2007) and effect of AMV on the growth characters of *A. hybridus* (Ehinmore and Kareem, 2010). However, information is lacking on the effect of TeMV on the nutritional quality of this all important vegetable. This work is therefore aimed at documenting the reaction of *Amaranthus hybridus* to infection by TeMV.

MATERIALS AND METHODS

Collection of seeds and planting: Seeds of *A. hybridus* were obtained from Akparabong market in Ikom Local Government Area of Cross River State (CRS), Nigeria. They were sun-dried for two days to enhance germinability and thereafter scattered on a steam-sterilized fertile garden soil in 16 cm diameter polyethylene bags. The seeds germinated and were inoculated with TeMV at the four leaf stage. The inoculum was prepared from diseased leaves of *T. occidentalis* and applied on *A. hybridus* by the conventional leaf-rub method (mechanical or sap inoculation) after application of carborundum (800 mesh). The inoculated leaves were then rinsed with water and left for symptom development while the control plants were only inoculated with the buffer.

Experimental design and inoculation of experimental plants: Test plants were arranged in two groups each containing 30 plants each which were set out in three rows of ten plants. Prior to inoculation, plants to be inoculated with the virus and those of controls in both groups were arranged in a randomized complete block design in the greenhouse with average temperature of 25±3°C. The inoculation of the test plants was as described above. Within each plant groups, fifteen were inoculated with the virus and the remaining fifteen inoculated with buffer only to serve as controls. The set up was monitored for symptom expression (8-10 days post-inoculation), which included mosaic, severe leaf malformation and distortion characteristic of TeMV infected *A. hybridus.* At three months period of development, infected and healthy leaves were harvested, oven-dried, grinded into fine powder and used to determine infection of TeMV on the nutritional quality of *Amaranthus hybridus*.

Sample analysis: The proximate compositions of (Moisture, crude protein, ash, lipid and fibre) of the samples were determined by the method of the Association of Official Analytical Chemists (AOAC., 1984). Mineral contents (Ca, Fe, Mg, Zn, Cu and P) were determined using atomic absorption spectrophotometer as outlined in AOAC (1984). Sodium and K were estimated by flame photometry. The antinutrients: hydrocyanate was determined according to AOAC (1984), phytate (Abara *et al.,* 2000) and oxalate (Dye, 1956) as described by Abara *et al.* (2000). Vitamin A content was determined spectrophotometrically using the hexane method. Vitamin C by modified method of Bessey (1944), vitamin B_1 (Thiamine) and vitamin B_2 (riboflavin and nicotinamide by AOAC (1984). While, amino acids were determined using methods described by Spackman *et al.* (1958).

Statistical analysis: The data obtained in this study was analyzed using the Student t-test. Results were also expressed as percentage difference and differences between mean values were determined at 5% probability.

RESULTS

The results of this study revealed that TeMV infection of *A. hybridus* caused significant reductions (p<0.05) in fibre, fat and protein contents when compared to the values obtained for the healthy controls (Table 1). The mean values of 4.23±0.2, 2.34±0.2 and 12.10±0.1 (g/100 g) were recorded for fibre, fat and protein respectively in infected sampled with corresponding mean values of 8.61±0.1, 4.65±0.01 and 17.92±0.2 (g/100 g) for control samples. However, results obtained for ash, moisture and carbohydrate showed marginal reductions when compared to the healthy controls. The percentage reduction in the content of these proximate composition engendered by TeMV ranged from 2.15% for carbohydrate to 49.68 for fat.

Results as presented in Table 2 highlight the effects of TeMV on mineral composition of *A. hybridus* infection of *A. hybridus* of resulted in significant decreases in all the elements with the exception of potassium. Significant decreases obtained for Na, P, Fe, Zn, Mg, Cu and Ca in infected samples were 0.07±0.02, 12.11±0.1, 6.21±0.02, 2.79±0.01, 0.02±0.01 and 29.21±0.1 mg/100 g,

Table 1: Effect of *Telfairia mosaic virus* proximate composition

Proximate composition	Infected (g/100 g)	Healthy (control) (g/100 g)	Difference (%)
Protein	12.10±0.10*	17.92±0.20	32.5
Fibre	4.23±0.02*	8.61±0.10	50.9
Fat	2.34±0.20*	4.65±0.01	49.7
Carbohydrate	92.13±0.02	90.13±0.30	2.2
Ash	13.01±0.01	13.80	5.7
Moisture	84.30±0.20	86.80	2.9

Values are Mean±SD, n = 3, *Significant at p<0.05

Table 2: Effect of *Telfairia mosaic virus* on mineral composition of leaves of *Amaranthus hybridus*

Mineral composition	Infected (mg/100 g)	Healthy (control) (mg/100 g)	Difference (%)
Sodium	0.07±0.02*	6.80±0.02	99.0
Potassium	47.40±0.10	53.01±0.10	10.6
Calcium	29.21±0.10*	43.32±0.03	32.6
Magnesium	129.15±0.10*	230.10±0.10	43.9
Iron	6.21±0.02*	14.58±0.20	57.4
Zinc	2.78±0.01*	3.90±0.10	51.3
Copper	0.02±0.01*	0.03±0.01	33.3
Phosphorus	12.11±0.10*	32.51±0.10	62.8

Values are Mean±SD, n = 3, *Significant at p<0.05

Table 3: Effect of *Telfairia mosaic virus* on the anti-nutrient composition of *Amaranthus hybridus*

Toxicants	Infected (mg/100 g)[a]	Healthy control (mg/100 g)[b]	Difference (%)[c]
Total oxalate	54.79±0.01*	38.82±0.02	41.4
Soluble oxalate	48.51±0.01*	31.79±0.01	52.6
Hydrocyanide acid	3.13±0.10*	1.57±0.01	49.8
Phytic acid	80.10±0.02*	120.30±0.30	33.4

Values are Mean±SD, n = 3, *Significant at p<0.05, Values were obtained by expressing the difference between the values for the healthy and the infected plant as a percentage of the healthy

Table 4: Effect of *Telfairia mosaic* virus on vitamins composition of *Amaranthus hybridus*

Vitamins	Healthy (mg/100 g)[a]	Infected (mg/100 g)[a]	Difference (%)[b]
Vitamin A (β-carotene)	4.51±0.010	2.01±0.03*	55.4
Vitamin C (ascorbic acid)	23.60±0.030	13.44±0.02*	43.1
Vitamin B_1 (thiamine)	3.21±0.010	2.10±0.02*	34.6
Vitamin B_2 (riboflavin)	1.73±0.002	1.27±0.01*	26.6
Vitamin B_3 (niacin)	1.60±0.012	1.39±0.02*	13.1
Vitamin B_6 (pyridoxine)	2.30±0.012	1.89±0.02*	17.83
Vitamin E (α-Tocopherol)	0.41±0.030	0.30±0.01	4.5

[a]Values are Mean±SD, n = 3 replicates, *significant at p<0.05, [b]Values were obtained by expressing the difference between the value for the healthy and the infected sample as a percentage of the healthy

respectively. Corresponding values obtained for healthy samples were 6.80±0.02, 32.51±0.1, 14.58±02, 3.90±0.1, 0.03±0.01, 43.32±0.03 (mg/100 g). Percentage reduction values for mineral elements ranged from 10.58 for K to 98.97% for Na.

Effects of TeMV on antinutrient contents of *A. hybridus* were found to be significantly higher in infected samples compared to the healthy controls with the exception of phytic acid. The mean values obtained for total oxalate, soluble oxalate and hydrocyanide acid in infected samples were 54.79±0.01, 48.51±0.01 and 3.13±0.1 (mg/100 g), respectively while, corresponding values for the healthy controls were 38.82±0.02, 31.79±0.01 and 1.57±0.01. Infection of TeMV on *A. hybridus* caused significant reduction (p<0.05) in phytic acid with mean value for infected sample of 80.10±0.02 and values for healthy sample of 120.30±0.3 (mg/100 g), respectively. Values for percentage difference in anti-nutrient ranged from 33.4% for phytic acid to 52.6% for soluble oxalate (Table 3).

The results of virus infection on the vitamin contents are presented in Table 4. Except for vitamin E (α-Tocopherol), which had comparable value with healthy controls, values for vitamin A, C, B_1, B_2, B_3 and B_6 were significantly reduced by the virus. The mean values obtained for vitamin A, C, B_1 B_2, B_3 and B_6 ininfected samples were 2.01±0.03, 13.44±0.02, 2.10±0.02, 1.27±0.01, 1.39±0.02 and 1.89±0.02 mg/100 g, respectively. Corresponding values for healthy samples were 4.51±0.01, 23.60±0.03, 3.21±0.01, 1.73±0.002, 1.60±0.012 and 2.30±0.012 mg/100 g. Percentage difference values in vitamins ranged from 4.5% for vitamin E to 55.4% for vitamin A.

Results as presented in Table 5 show significant (p< 0.05) decline in sulphur containing amino acids: cysteine, isoleucine, leucine, lysine and methionine with a range in percentage decline from 11.8% (leucine) to 35.3% (methionine). Phenylalanine and tyrosine (aromatic amino acids) also showed decline due to TeMV infection. Nonessential amino acids (glutamic acid, aspartic acid and proline) were significantly higher in infected samples. Percentage reductions due to infection of TeMV on *Amaranthus hybridus* for essential and nonessential amino acids ranged from 5.0% (tyrosine) to 35.3% (methionine). The significant decline in essential amino acids (histidine, lysine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine) and nonessential amino acids (alanine, serine, arginine, cysteine, tyrosine and glycine) and increase in infected samples of glutamic acid, aspartic acid and proline.

Table 5: Effect of *Telfairia mosaic* virus on amino acids profile of *Amaranthus hybridus*

Amino acids	g/16 N Inoculated[a]	Healthy[a]	Difference (%)[b]
Cysteine	0.24±0.06*	0.47±0.03	31.9
Isoleucine	2.55±0.1*	3.44±0.03	25.9
Leucine	6.03±0.1*	6.84±0.10	11.8
Lysine	2.58±0.06*	3.12±0.03	17.3
Methionine	0.99±0.06*	1.53±0.03	35.3
Arginine	3.03±0.06*	4.10±0.06	26.1
Alanine	3.61±0.1	3.30±0.06	9.4
Threonine	2.56±0.03*	3.01±0.06	15.0
Histidine	2.01±0.1	2.19±0.10	8.2
Valine	3.11±0.1*	5.01±0.20	34.1
Proline	3.89±0.1	3.58±0.03	0.7
Glutamic acid	17.96±0.03	14.99±0.1	19.8
Aspartic acid	7.25±0.03	6.62±0.10	9.5
Glycine	3.02±0.03*	3.91±0.03	22.8
Serine	2.82±0.06	3.08±0.06	8.4
Tyrosine	2.95±0.03	3.11±0.06	5.1
Phenylalanine	3.69±0.03*	4.20±0.03	12.1

[a]Values are Mean±SD, n = 3 replicates, *Significant at $p<0.05$, [b]Values were obtained by expressing the difference between the value for the healthy and the infected sample as a percentage of the healthy

DISCUSSION

The reaction of *A. hybridus* to TeMV infection resulted in caused significant ($p<0.05$) reductions in fibre, fat and protein contents, all the mineral elements with exception of K, vitamins A, C, B_1, B_6 and B_3 with significant increases in anti nutrients with the exception of phytic acid in infected samples when compared to healthy ones. Previous reports of virus infection on protein contents are varied and seem depended on host-virus combination. Muqit *et al.* (2007) reported a decrease in the protein content of *Benincasa hispidia* infected with *Bottle gourd mosaic virus* (BgMV) and *Watermelon mosaic virus*-2 (WMV-2) singly. Mofunanya *et al.* (2008) have also reported a decrease in the protein content to *T. occidentalis* inoculated with TeMV. Owolabi *et al.* (2010) observed a decrease in protein content in Ivy gourd infected by a Nigeria strain of *Moroccan Watermelon Mosaic Virus* (MWMV). A decrease in carbon, nitrogen and protein in *Daucus carota* due to infection of *Cucumber mosaic virus* and increased P in diseased plant has been reported by Afreen *et al.* (2011). *Soya bean mosaic virus* (SoyMV) and *Cowpea Chlorotic Mottle Virus* (CCMV) (Demski and Jellum, 1975), *Potato Virus* X (PVX) and Potato virus Y(Gommaa *et al.*, 2004) and *Tobacco Mosaic Virus* (TMV) (Barka and El-Maaty, 2008) have been reported to cause decreases in the protein contents in soybean, *Nicotiana tabacum* and tomato, respectively. Results of this study however, contrasts with those of White and Blakke (1992), who reported increased level of protein in barley infected with WSMV and BSMV separately. Milavec *et al.* (2001) and Hemida (2005) have also documented increases in the protein content in potato-PVY^{NTN} and *Vicia faba*-BYMV combination. Significant reductions in fibre and fat observed in this study is consistents with those of Mofunanya *et al.* (2008) in *T. occidentalis*-TeMV combination and Owolabi *et al.* (2010) in Ivy gourd-MWMV combination.

Reductions in mineral elements recorded for Na, P, Fe, Mg, Cu and Ca in this study correspond to previous reports by Mofunanya *et al.* (2008) and Owolabi *et al.* (2010) but differs from that of Nogueira *et al.* (1996) who reported increased level of Fe, while the level of Mg was similar to that of the control. Salama *et al.* (1975) and Shattuck (1987) have reported increased levels of P in infected leaf tissues of broad bean and *Brassica napus* spp. Rapifera infected with *Broad bean mosaic virus* (BBMV). Owolabi *et al.* (2010) recorded significant reductions in Mg, Fe and Ca with increases in P, Mn and K in Ivy gourd leaves (*Coccinia barteri*) infected with a Nigerian strain of MWMV.

Significant increase in the contents of total oxalate, soluble oxalate and hydrocyanide acid obtained in infected samples in this study agrees with previous reports by Mofunanya *et al.* (2008). The reductions in vitamins in TeMV infected *A. hybridus* correspond to report of reductions in vitamin A and C in *T. occidentalis* infected by TeMV. Mofunanya *et al.* (2008, 2009), Owolabi *et al.* (2010), Raj *et al.* (2005) and Prakash *et al.* (2006) and Kapinga *et al.* (2009) also reported losses in these vitamins in other plant-virus combination.

Reductions in amino acids profile due to TeMV infection of *A. hybridus* in this study are in consonance with results of previous reductions resulting from TeMV infection in *T. occidentalis* ecotypes (Mofunanya *et al*., 2009). Mofunanya *et al*. (2009) reported a decrease in infected samples of essential and nonessential amino acids with increase in infected samples of glutamic acid, aspartic acid, valine and proline. Reductions by BYMV in *Vicia faba* and *Phaseolus vulgaris* (Hemida, 2005) and MWMV in leaves of Ivy gourd (Owolabi *et al*., 2010). The decrease in amino acids may be due to their utilization by the virus and enhanced enzyme activities in leaves while increase in these amino acids which are protein constituents may be due the fact that the coat protein of a virus in this case TeMV may come to represent about half the protein in disease leaf (Hull, 2002).

Accumulation of proline and other amino acids is a common metabolic response of higher plants to both abiotic and biotic stress, many plants accumulate high amount of proline in their tissues (Drossopoulos *et al.,* 1985; Mazid *et al*., 2011).

The presence of these amino acids in *A. hybridus* agrees with results of Akubugwo *et al*. (2007). Seventeen instead of twenty amino acids were determined. This was due the conversion of asparagines and glutamine to glutamic acid and aspartic acid respectively and the destruction of tryptophan that occur during hydrolysis (Wathelet, 1999). The reductions in these minerals could be due to the possible adverse effects and alteration in plant metabolism induced by viral infection.

Plant nutrients are vital to keep the body healthy and alive. They are needed to build and repair cells and tissues of the body as well as maintenance of the organs and bone in working optimally and to provide warmth, energy and fuel. Eating food rich in nutrients can help in preventing common ailments and life threatening disease and illness (Premier, 2002). Adewale and Olorunju (2013) reported that the cheapest and readily available sources of proteins, vitamins, minerals and amino acids are vegetables.

The significant reductions in proximate, mineral elements, vitamins and amino acids profile caused by the reaction of *A. hybridus* to TeMV infection reduced the nutritional value of this all important vegetable where large quantities are consumed daily by the Nigerian populace. These reductions should be looked into jealously because consumption of infected leaves is of least significance as it has just lower amount of the required nutrients when compared to healthy leaves. The use of resistant variety as a practical means of controlling the virus and biotechnological research that will result in the production and deregulation of virus-resistant *A. hybridus* through coat protein gene transfer should be intensified.

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