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The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed proteins

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Abstract

The possibility of using electrophoresis to characterize varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* cultivated in Nigeria was investigated. The SDS-polyacrylamide gel electropherogram of extracted total seed proteins of 10 breeding lines in each of the 6 varieties investigated, revealed a pattern in which 12 polypeptide bands with apparent molecular weight range of 22 to 98 kilodaltons could be distinguished. The result showed that the six varieties could be characterized on the basis of presence/absence and staining intensities of 7 polypeptide bands. It is suggested that SDS-polyacrylamide gel electrophoresis of seed proteins provides a useful analytical technique for the characterization of varieties of pepper and there may be genotype duplicates in the collection of Nigerian *Capsicum* germplasm.

Introduction

Pepper (*Capsicum* spp.) is an important vegetable and spice crop grown in tropical, subtropical as well as temperate regions. Peppers are rich in vitamin C and the pungency of the fruit is due to the presence of capsaicin (Walter, 1986). The genus *Capsicum* comprises five cultivated species, of which few are worldwide in distribution, and approximately twenty wild species (Walter, 1986). In Nigeria, the genus is represented by two cultivated species (*Capsicum annuum* L. and *Capsicum frutescens* L.) comprising six varieties. Four of the six varieties are of *Capsicum annuum* – namely vars. *abbreviatum* Fingerh., *grossum* Sendt., *jalapeno* Miller and *longum* Sendt – and two are of *Capsicum frutescens* – namely vars. *maxima* Roxb. and *minima* Roxb. Five of the varieties (*Capsicum annuum* vars. *abbreviatum, grossum, longum* and *Capsicum frutescens* vars. *maxima* and *minima*) are called hot peppers because of their high pungency while *Capsicum annuum* var. *jalapeno* is called sweet pepper because of its low pungency. However, jalapeno peppers have fruit characters which are relatively unreliable taxonomically and are thus sometimes considered as hot peppers. Members of the genus are diploids (2n = 24) and are either annuals or perennial. The different species are usually distinguished morphologically by a combination of flower and fruit characteristics. *Capsicum annuum* has white flowers, blue to purple anthers, a toothed calyx and typically single-fruited nodes while *Capsicum frutescens* has greenish flowers, a non-toothed calyx, blue flowers and mostly single fruited nodes (Daskalov, 1986). Most of the varieties or cultivars within a species can be distinguished at the fruiting stage by the shape and size of the fruits. However it has been osbserved that locational differences affect fruiting, thus there is a problem with the morphological characterization of cultivars of *Capsicum* species. For these reasons attention has turned to biochemical methods for cultivar characterization because such methods are more sensitive and can eliminate or greatly reduce environmental effects (Cooke, 1984).

As a first measure to improve the quality factors of cultivars, it is necessary to screen existing cultivars, breeding lines and germplasm collections using

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simple, quick and cheap screening techniques. Electrophoresis of seed proteins and enzymes is a powerful tool for distinguishing between cultivars and analyzing species relationships or agriculturally important crops (Ladizinsky & Hymowitx, 1979; Cooke, 1984, 1988). Cereal species have been extensively investigated, to such extent that electrophoresis is widely used to check the pedigree or varietal identity and seed purity of the cereal grain in trade for the purposes of registration, plant variety and utility patents (Wrigley et al., 1982; Smith, 1988; Yupsanis et al., 1992). Other crops have not received such extensive and intensive investigations as cereals. In legume crops there are several reports on the characterization of cultivars, varieties and species of peas, beans and cowpea using various seed protein electrophoretic methods (Goodrich et al., 1985; Cooke, 1988; Oghaiake et al., 1993; Odeigah & Osanyinpeju, 1996). Seed protein electrophoresis has also been used as markers in the characterization of other crops. Chen et al. (1990) investigated 27 *Gossypium* (cotton) species and found that seed protein banding patterns were similar within species but differed between genomes. Datta et al. (1992) evaluated the electrophoretic seed protein profiles of control and mutant lines of *Sesamum indicum* L. by polyacrylamide gel electrophoresis. The results revealed dissimilar seed protein profiles of band types, band number and relative mobility values for the control and mutant lines.

Eight varieties of dill (*Anethum graveolens* – an important medicinal plant, also used as a herb for flavouring) have been characterised by electrophoresis (Manju et al., 1993). There was significant variation between varieties in seed protein content and protein profiles were also qualitative different between varieties.

Enzymatic (especially isozyme electrophoresis) and RFLP (restriction fragment length polymorphism) markers have also been used in cultivar identification (Smith, 1988; Singh et al., 1991; Williams et al., 1993). There are relatively less of these investigations in vegetable species. Suurs et al. (1989) used a refined method of isozyme electrophoresis to obtain reproducible banding patterns for variants of genetic markers in *Solanum* and *Lycopersicom* species.

Pepper (*Capsicum* spp.) is grown worldwide and it is the second most cultivated vegetable species after tomato in the third world. However, to date there are very few cultivar identification studies using biochemical markers in pepper. Conicella et al. (1990) carried out cytogenetic and isozyme studies of wild and cultivated *Capsicum annuum* and their work suggested that a cultivated plant and its immediate progenitor still share more or less the same protein profile. Belletti et al. (1992) have also studied allozyme variability in *Capsicum*. RFLP techniques have also been used to study French and Mexican accessions of *Capsicum* (Lefebvre et al., 1993, 1995; Prince et al., 1992).

In order to have a comprehensive *Capsicum* gene list (Daskalov & Poulos, 1994), it is essential that the accessions from various countries, particularly the third world be characterized. There do not appear to have been any investigations on the use of electrophoresis on varietal identification of Nigerian accessions of *Capsicum*. We report herein an investigation on the use of seed protein electrophoresis in the characterization of varieties/species of *Capsicum* (pepper) cultivated in Nigeria.

Materials and methods

Plant material

Seed of mature fruits of the six varieties of pepper were collected from the fields of the National Institute of Horticulture (NIHORT) Ibadan, South-Western, Nigeria. Ten-seed sample (about 0.5 g) from the same fruit was collected from one line and ten lines per variety were analysed for this study. One-seed samples could not yield a homogenate with enough protein for electrophoretic analysis.

Extraction seed proteins

The ten seeds from each line were ground to a fine powder using a mortar and pestle and 100 mg of the extract was de-fatted by washing with three changes of cold acetone for 4–6 h. The acetone was removed by filtration and samples were air-dried at room temperature. The de-fatted meal was extracted with two sequential 2 ml aliquots of 0.05 m Tris-glycine buffer pH 8.0 for 2 h at 4 °C. The total seed protein fraction (supernatant) was recovered by centrifugation at 5,000 rpm for 30 min at 4 °C and 20 μl aliquots were used immediately for electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

10% SDS-polyacrylamide slab gel electrophoresis was carried out using a discontinous gel technique as described previously (Odeigah & Osanyinpeju, 1996). The gels were removed, stained for 30 min with

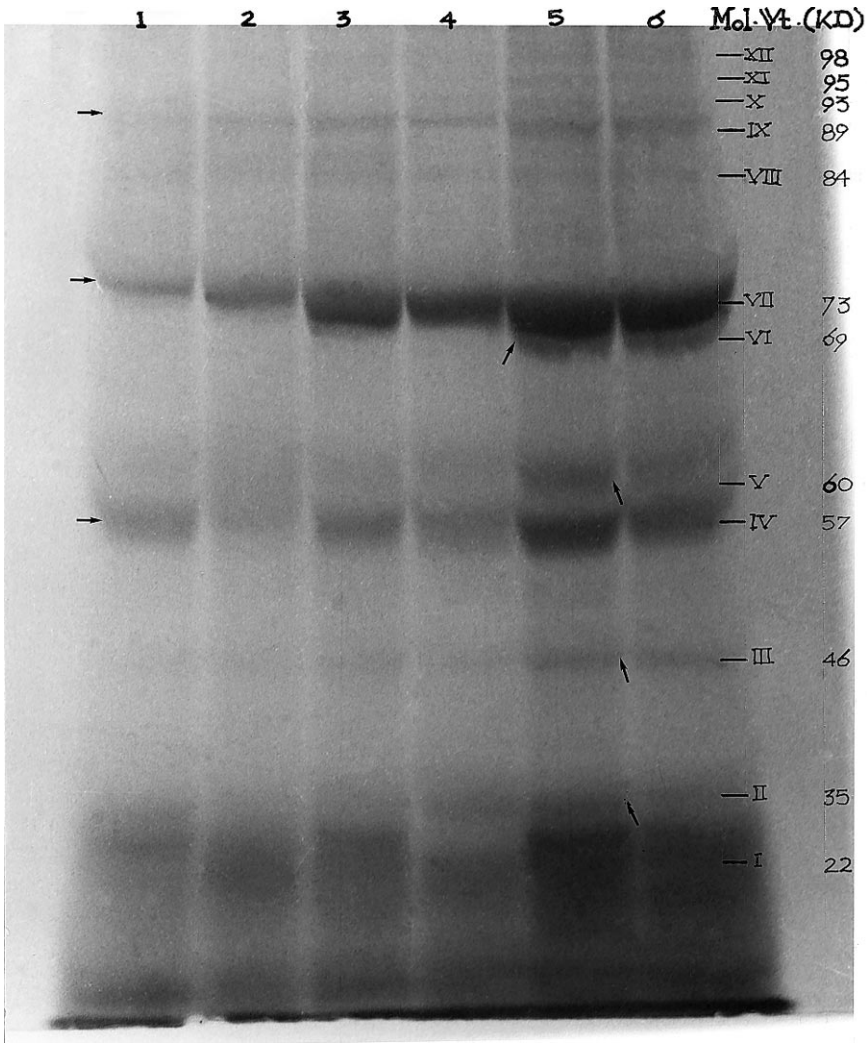

Fig. 1. Electropherogram of total seed proteins of six varieties of pepper. Lane 1. *Capsicum annuum* var. *abbreviatum*; Lane 2. *Capsicum annuum* var. *grossum*; Lane 3. *Capsicum annuum* var. *longum*; Lane 4. *Capsicum annuum* var. *jalapeno*; Lane 5. *Capsicum frutescens* var. *maxima*; Lane 6. *Capsicum frutescens* var. *minima.* (observed differences between varieties are highlighted by arrows). (No differences were observed in the banding pattern of the 10 lines within a variety, so only varietal electropherograms are shown in this figure).

2% (W/V) coomassie blue in an ethanol-water-glacial acetic acid mixture (45 : 45 : 10) at 60 °C, destained for 30 min in a similar solvent mixture (65 : 25 : 10) and left overnight in 7% (V/V) acetic acid at room temperature for complete destaining. Estimation of the molecular heights of the polypeptides was carried out according to the method of Weber and Osborn (1969) using bovine serum albumin Mol. Wt. 67,000; ovalbumin, Mol. Wt. 43,000, chymotrypsinogen, Mol. Wt. 27,000 and rihonuclease Mol. Wt. 13,700 daltons as markers. The electrophoresis was repeated three times with different seed lots for the same line of a variety.

Results

The banding patterns among the lines within a variety were repeatable with different seed lots and the results did not show any intra-variety differences for all lines within a botanical variety for the six varieties of *Capsicum* spp. investigated.

The SDS-polyacrylamide gel electropherogram of seed proteins of the six varieties of pepper investigated is shown in Fig. 1. As can be seen, twelve polypeptive bands designated I to XII with apparent molecular weight range of 22 to 98 kilodaltons (KD) could be distinguished. The molecular weights of the

bands were determined by reference to the mobilities of the markers and the values are intended to facilitate the identification of bands rather than to establish precise values for the molecular size of each component. The polypeptide bands vary considerably with respect to their staining intensities in all the varieties. On the basis of the intensity of coomassie blue staining four major components (bands I, IV, VI, VII with apparent molecular weights of 22, 57, 69 and 73 KD respectively) could be identified. Three components (bands II, III and V with molecular weights of 35, 46 and 60 KD respectively) were intermediate in staining intensities while others (bands VIII to XII with molecular weight range of 84 to 98 KD respectively) were minor components as judged by staining intensities. Bands X to XII are more like traces while band I is a heavy band which may be more than one component judging by the width of the band.

There are great similarities in the overall polypeptide profiles of the seed proteins from the six varieties. However, the analytical system revealed moderate and repeatable differences in the varieties. Using the presence or absence of some bands and also differences in staining intensities of bands it was possible to separate the varieties into distinguishable groups. The two varieties of *Capsicum frutescens* (Lanes 5 and 6) can be separated from the four *Capsicum annuum* (Lanes 1 to 4 of Fig. 1) by the presence of polypeptide bands II (35 KD), V (60 KD) and VI (69 KD). Differences in the polypeptide bands between varieties within the same species appear to be more of staining intensity than of presence or absence. For example *Capsicum frutescens* var. *maxima* (lane 5 of Fig. 1) can be distinguished from *Capsicum frutescens* var. *minima* (lane 6) by differences in the staining intensities of bands II, III, and V. Similarly varieties of *Capsicum annuum* (lanes 1 to 4) can be distinguished by differences in the staining intensities of bands IV, VII and IX respectively. There was no positive correlation between the presence or absence of specific polypeptide bands and pungency or any specific characteristis of the crop in the varieties analysed. However the sweet pepper, *Capsicum annuum*, var. *jalapeno* (lane 4) appears to have a trace of band II which is present only in varieties of *Capsicum frutescens.*

Discussion

To facilitate germplasm improvement and documentation of any crop, it is necessary to screen cultivars, breeding lines or accessions in order to have an inventory of the genetic variation in the crop. Proteins as primary gene products are good markers of genetic variation. The number of varieties investigated by this study is small. This is because the objective of the study was to screen varieties of pepper grown in Nigeria in order to document or assess the genetic variability or diversity in the crop. This information is necessary for subsequent crop improvement programmes of the germplasm. The results of investigating ten lines per variety and the absence of intra-variety differences in banding pattern even between different seed lots for the same line suggests varietal homogeneity or purity. The results of this preliminary study have shown that electrophoresis of seed proteins provide a possible and useful molecular analytical method for characterizing varieties of pepper. By using the presence/absence and differences in staining intensities of polypeptide bands, the varieties studied could be differentiated. Coomassie blue is a quantitative stain, thus differences in the relative staining intensity of the bands suggest that the quantitative representation of each component is not the same in all the varieties.

Seed protein isozyme electrophoresis and other biochemical techniques have been used for varietal or cultivar identification in other crops such as maize (Smith, 1988), wheat (William et al., 1993), rice (Yupsanis et al., 1992), cotton (Chen et al., 1990) and legumes such as the common bean (Singh et al., 1991) and cowpea (Oghaiake et al., 1993; Odeigah & Osanyinpeju, 1996).

Most of the biochemical characterization studies of cultivars and varieties of vegetable crops such as *Solanum*, *Lycopersicon* and *Capsicum* species have used mainly enzymatic and RELP markers. The results of this study have shown that seed protein markers also provide useful analytical techniques for varietal characterization in pepper.

No differences were observed in the polypeptide patterns of the breeding lines with a variety despite the morphological diversity. It seems therefore that there exists genotype duplicates in the collection of Nigerian *Capsicum* germplasm. Seed samples of *Capsicum* collected from major markets across the country during a germplasm exploration were the same as those obtained from NIHORT. This confirms the fact that NIHORT is the major repository of *Capsicum* germplasm in the country.

This study suggests that there is not much genetic diversity in Nigerian *Capsicum* germplasm. The genetic diversity can be improved by mutation breeding

to produce new mutant cultivars or the introduction of new varieties from other countries. Work is in progress to produce mutant cultivars of *Capsicum* by mutation breeding. Mutant lines so far obtained are being evaluated in multi-locational trials. It is hoped that the results of this study would facilitate the documentation of the genetic diversity in Nigerian *Capsicum* germplasm and help in the update of the official *Capsicum* gene list publication (Daskalov & Poulos, 1994).

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