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Genetic diversity and population structure ofmodified three-way tomato hybrids for determining fruit size traits

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Abstract: The assessment of genetic diversity and genetic structure of crops has a vital impact on the plant breeding program, including the characterization, use and conservation of genetic material. The genetic diversity and genetic structure of a set of 94 F_3 tomato hybrids were assessed with 25 polymorphic SNP markers of quantitative trait loci (QTLs) underlying fruit size in tomatoes using Sequenom Mass ARRAY system. A total of 50 alleles were amplified and the average polymorphic information content (PIC) was 0.2220. Nei's genetic distance ranged from 0 to 0.62. Therefore, single nucleotide sequence (SNP) markers detected a significantly high degree of polymorphism in tomato hybrids. Cluster analysis using the unweighted pair group method with arithmetic mean methods (UPMA) indicated that the tomato hybrids studied are grouped into four main groups, which is to some extent consistent with the size, shape and number of locules of the tomatoes studied. Analysis of population structure with SNP markers revealed three subpopulations. Association mapping using 25 SNP markers detected 9 markers with a significant association with mean fruit weight, fruit length, fruit diameter, number of locules, and fruit shape index. The nine markers detected in this study are recommended for the tomato fruit size improvement breeding program.

Keywords: Genetic diversity, Population structure, SNPs markers, *Solanum lycopersicum*.
Abbreviations: BF_Beef (Florida); DNA_Deoxyribonucleic Acid; QTL_Quantitative trait loci; PIC_polymorphic information content; PR_Plumb (Rio grande); S_Supersteak; SNPs_Single nucleotide sequence; UPGMA_Un-weighted Paired Group Method of Arithmetic Averages.

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important local market vegetables in Nigeria grown by small-scale farmers.Tomato production is considered as one of the main agricultural enterprises as it employs people in farms, processing industries and provides higher income per hectare to small holder farmers than most staple crops (AVRDC, 2006). However, they are many constraints affect the productivity and quality of tomato. Some of such constraints are high humidity and rainfall and lack of locally adapted cultivars.

The main objectives of current tomato breeding, such as increasing fruit size, require a good understanding and management of the diversity of cultivated genetic resources (Xu et al., 2013). Interpreting patterns of genetic variability in cultivated landraces of economically important crops allows breeders to reconsider this trait reservoir and, eventually, identify new alleles to improve productivity, adaptation, fruit quality and size, and nutritional value. To date, much of this germplasm has not been widely characterized and most local varieties have not yet been used in modern plant breeding (Huang et al., 2010).

The improvement of tomato with an ability to withstand the high humidity conditions of South Eastern Nigeria impelled the initiation of a hybridization programme. Crosses between two commercially acceptable but poorly adapted cultivars, Roma VF and Tropica and wild variety produced tomato hybrids with abundant fruiting (Atugwu and Uguru, 2012) and increased disease resistance (Uguru and Igili, 2002) under high rainfall conditions. However, the average fruit size of the tomato hybrids generated did not meet the level of acceptability in the local market. This would necessitate further crosses between the hybrids with exotic breeds with large fruited inbred (supersteak) which called a modified three way crosses and the selection from the segregating population. Successive evaluations of the progenies at different filial generation from F_1 toF_2showed reliable evidence of increased fruit yield particularly in terms of fruit size. Since fruit size is quantitatively inherited, that mean affected by environment, the molecular markers analysis is inevitable to confirm the fruit size quantitative trait loci incorporated in the tomato hybrids resulted from the modified three way crosses.

Successful breeding for crop improvement programmes depends on genetic variability that arises from genetic diversity (Ranaand Bhat, 2004). Lack of genetic variability may limit breeding progress and gain from selection (Cornelious

and Sneller, 2002). So, knowledge of the genetic diversity of any germplasm collection provides a basis for improvement of crops and development of superior cultivars. Detailed understanding of the population structure and diversity is also needed for the conservation planning, management and utilization of tomato germplasm (Hamrick and Godt, 1996; Frankham et al., 2002).

The availability of cost-effective, accurate and rapid genotyping tests has made single nucleotide polymorphism (SNP) the most frequently used DNA marker for high-throughput plant analysis, encouraging sequence variation analysis in germplasm collections. In different plant species, molecular data were used to infer the existence of a genetic structure in the study collection or to assign individuals to genetically differentiated groups that may be compatible with their breeding history (Mc Nailly et al., 2009).

Single nucleotide polymorphisms (SNPs) are known as a strong class of molecular markers that have immense significance in plant genetics and breeding because of their excellent distribution throughout the genome and suitability for genetic diversity analysis, evolutionary relationships and genetic population substructure estimation (Rafalski, 2002; Garris et al., 2003; Varshney et. al., 2008). Therefore, Single Nucleotide Polymorphisms (SNPs) markers of the quantitative trait loci (QTL) underlying fruit sizes in tomato were performed in this study. The present study aimed to investigate the genetic diversity and population structure of three way tomato hybrids with respect to fruit size determining traits.

Results

Single Nucleotide Polymorphisms (SNPs) diversity

The results from 25 SNPs markers used for this work on tomato fruit size detected appreciable degree of polymorphism within the set of tomato progenies used for this work. A total of 50 SNPs alleles were detected and the total number of allele detected per primer was 2. Most of the loci produced a maximum of two rare alleles. Some of the alleles may be useful as diagnostic markers for some of the assayed tomato progenies.

Most loci were highly polymorphic as indicated by values for polymorphism information content (PIC), expected heterozygosity or gene diversity (H_e) and observed heterozygosis' (H_o). The PIC value for each marker ranged from 0.0487 for the marker detected by Solyc2 - 2 to 0.3749 for the marker detected by Solyc4 – 1 (Table 1). The markers PIC value greater than 0.5 were considered highly informative and markers with 0.5 >PIC>0.2 were just considered to be informative (Bostein et al., 1980). However, because of the bi allelic nature of the SNPs markers, the maximum PIC value is only 0.5 while SSRs markers can go beyond 0.5. The variation was significantly associated with the number of alleles detected at each locus. Therefore, SNPs markers showed a reasonable amount of variation in the tomato genotypes in this study.

The expected heterozygosity or gene diversity (He) value per marker ranged from 0.05 at Solyc2 - 2 to 0.499 at Solyc4-1 with the mean value of 0.266 (Table 1). The observed heterozygosities were ranged from 0.05 at Solyc2-2 to 0.372 at Solyc4-1. Overall, the expected heterozygosities were higher than the observed heterozygosities. The major alleles

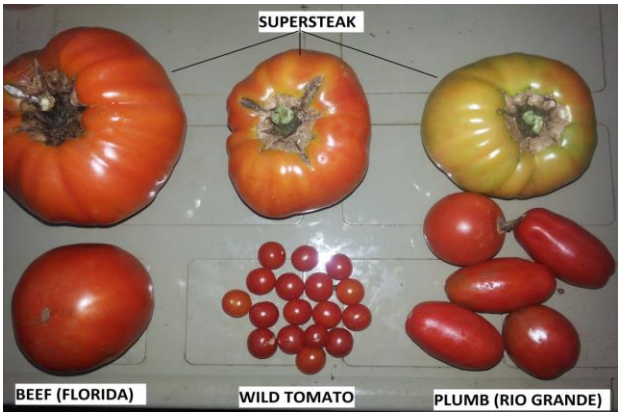

Figure 1. Tomato parents used in this study.

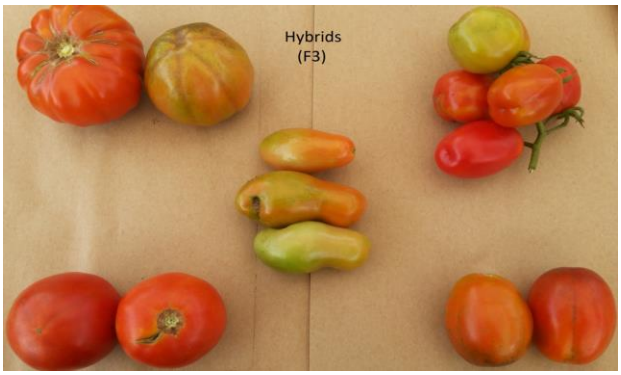

Figure 2: Variation in fruit size and shapes of the F_3 progenies develop from S x (W x R).

frequency for each marker ranged from 0.51 to 0.97; therefore the minor alleles were less than 0.5 in most of the markers.

The genetic distance among the tomato progenies in this study

Based on the information obtained at the 25 SNPs loci, Nei genetic distance coefficients were estimated for all pair-wise comparison of the tomato genotypes (F_3) developed from modified three- way cross between advanced generation tomato hybrids and Supersteak (Figure 2).

The average distance between individual tomato progenies was moderate at 0.56. The genetic distance among the F_2 tomato genotypes ranged from 0.0 to 0.6174 (Supplementary Table 3) while for F_3 ranged from 0.000 to 0.6154 (Supplementary Table 3). This value is an indication of the magnitude of diversity among the progenies studied. However, some tomato genotypes in both F_2 and F_3 showed 100% similarities.

The largest genetic distance was observed between S28 and S25 (0.6174), while the lowest one was detected between S33 and S30, S33 and S31 (0.000) for the F_2 tomato genotypes. For the F_3 tomato genotypes, the largest distance was observed between S63 and S55 (0.6154), while the lowest was detected between S64 and S55 (0.000).

Genetic diversity among tomato progenies using UPGMA-based cluster analysis

Tomato progenies were separated using un-weighted pair-group mean algorithm (UPGMA) dendrogram (Sneath and

Table 1. Allelic variation of 25 SNPs loci in the tomato hybrids.

Marker	MAF	G	A^{o}	Availability	He	Ho	PIC
Solyc04 - 1	0.5116	3.0000	2.0000	0.7167	0.4997	0.3721	0.3749
Solyc11 - 1	0.9375	3.0000	2.0000	0.9333	0.1172	0.0893	0.1103
Solyc11 - 3	0.9375	3.0000	2.0000	0.9333	0.1172	0.0893	0.1103
Solyc11 - 7	0.9167	2.0000	2.0000	0.5000	0.1528	0.1667	0.1411
Solyc11- 8	0.9286	2.0000	2.0000	0.5833	0.1327	0.1429	0.1239
Solyc11 - 9	0.8750	3.0000	2.0000	0.8667	0.2188	0.2115	0.1948
Solyc11 -10	0.8729	3.0000	2.0000	0.9833	0.2219	0.2203	0.1973
Solyc11 - 11	0.8878	2.0000	2.0000	0.8167	0.1993	0.2245	0.1794
Solyc11 - 12	0.6098	3.0000	2.0000	0.6833	0.4759	0.5366	0.3627
Solyc11 - 13	0.7045	3.0000	2.0000	0.7333	0.4163	0.3182	0.3297
Solyc11 - 14	0.7586	3.0000	2.0000	0.9667	0.3662	0.2759	0.2992
Solyc11 - 15	0.8298	3.0000	2.0000	0.7833	0.2825	0.2553	0.2426
Solyc11 - 16	0.7128	3.0000	2.0000	0.7833	0.4095	0.3191	0.3256
Solyc11 - 17	0.7364	3.0000	2.0000	0.9167	0.3883	0.3091	0.3129
Solyc11 - 19	0.7586	3.0000	2.0000	0.9667	0.3662	0.2759	0.2992
Solyc11 - 21	0.7800	3.0000	2.0000	0.8333	0.3432	0.2400	0.2843
Solyc02 - 1	0.5488	3.0000	2.0000	0.6833	0.4952	0.3171	0.3726
Solyc02 - 2	0.9744	2.0000	2.0000	0.6500	0.0500	0.0513	0.0487
Solyc11 - 2	0.9500	2.0000	2.0000	0.6667	0.0950	0.1000	0.0905
Solyc11 - 4	0.9386	2.0000	2.0000	0.9500	0.1153	0.1228	0.1086
Solyc11 - 5	0.9286	3.0000	2.0000	0.9333	0.1327	0.1071	0.1239
Solyc11 - 6	0.8600	2.0000	2.0000	0.8333	0.2408	0.2800	0.2118
Solyc11- 18	0.8191	2.0000	2.0000	0.7833	0.2963	0.3617	0.2524
Solyc11 -20	0.8491	2.0000	2.0000	0.8833	0.2563	0.3019	0.2235
Solyc11- 22	0.8426	2.0000	2.0000	0.9000	0.2653	0.3148	0.2301
Mean	**0.8188**	**2.6000**	**2.0000**	**0.8113**	**0.2662**	**0.2401**	**0.2220**

MAF = Major allele frequency, G = Genotype number, A^{o} = Number of allele, He = Expected heterozygosity/gene diversity, Ho = Observed heterozygosity, PIC = Polymorphism information content (PIC).

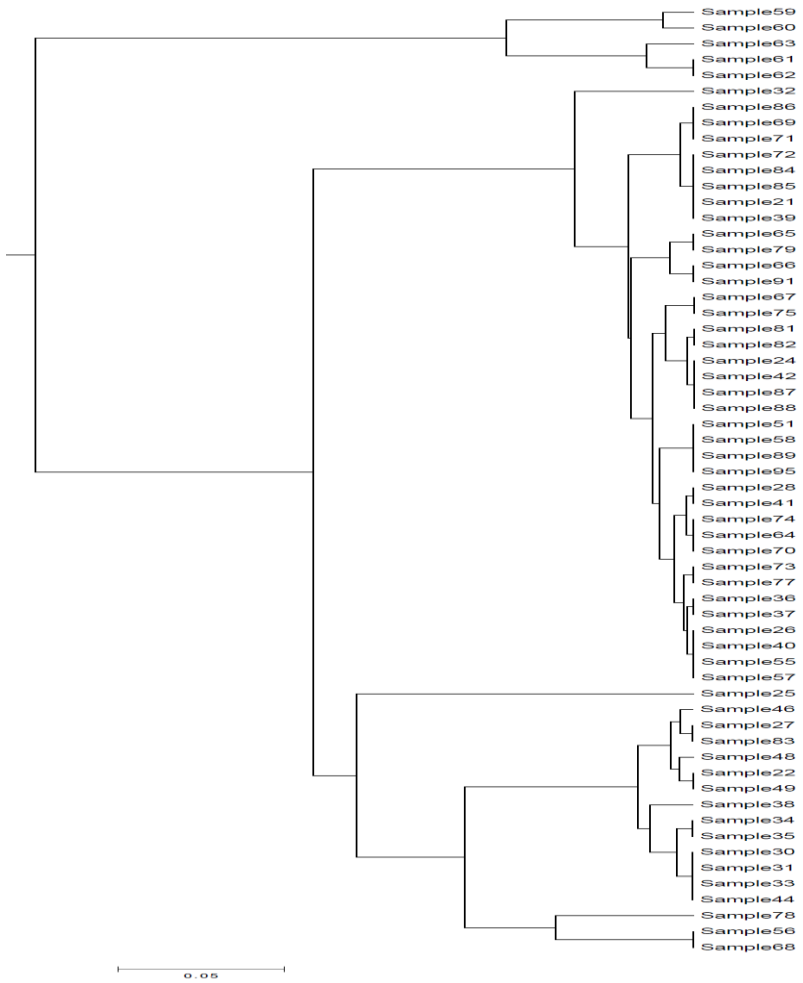

Figure 3. Dendrogram constructed using Nei similarity coefficient and UPGMA Clustering for the F_3 tomato genotypes.

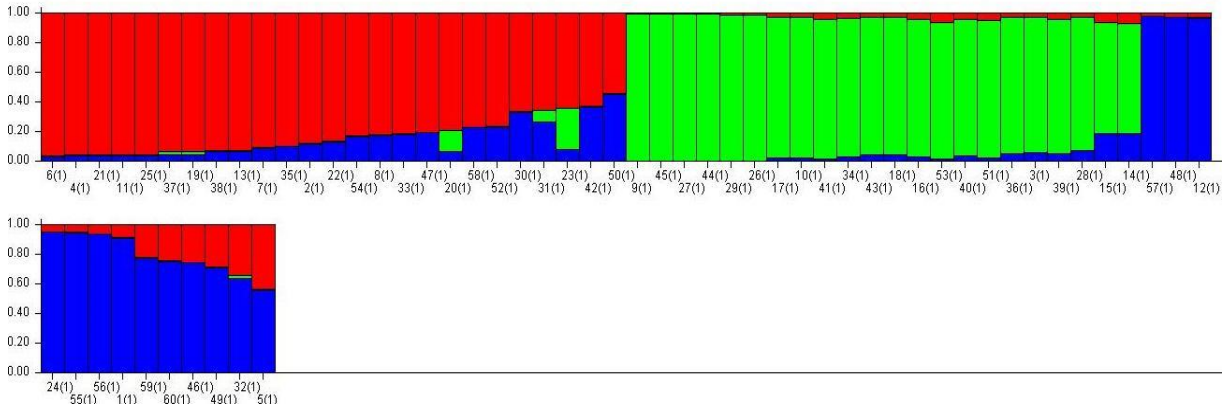

Figure 4. Each individual sample was represented by a single row broken into three-colored segments (red, green and blue), with length proportions to each of the two inferred population subgroups. Each individual corresponded to the samples in the dendrogram.

Sokal, 1973) to describe their genetic relationship. The similarities between the tomato progenies hybrids reflected in the 25 SNPs alleles were estimated and grouped into four major groups (Figure 3). The first cluster consists of tomato progenies with large fruit size, the mean locule number per fruit greater than 5 and the fruit shape index of less than 1. The members of this clusters showed a direct relationship with a Supersteak, one of the parents used in this work.

Cluster 2 comprised tomato progenies with locule number ranged from 2 to 3 and the fruit shape index around 1. The members of this clusters showed a direct relationship with the advanced generation hybrid that generated from wild tomato and roma VF. Cluster 3 comprised of tomato hybrid with locule

number ranged between 3 to 5 and fruit shape index around 1. Apart from three major groups, a number of hybrids were scattered and distributed across the clusters.

Population structure of the tomato progenies

Genotyping data generated using the 25 polymorphic SNP markers were used for genetic structure analysis using the Bayesian clustering model implemented in the structure software. DK was also calculated, and the result showed that DK reached the maximal value when K = 3. The model used indicated that K = 3 is the best number of sub-population (hereafter referred to as Q = 3, providing support for the existence of the three distinct clusters in our association panel. The analysis of these data identified accessions into three subgroups as well, and the results were very similar to those of the clustering results (Figure 4). The Q matrix outputs of the three subpopulations were used for the association analysis.

Discussion

SNP-based polymorphism and genetic diversity

Average Nei's gene diversity and Polymorphism Information Content (PICs) values revealed by SNP markers in this study were 0.2662 and 0.2220, respectively. This level of genetic diversity is similar to the report of Corradoet al. (2013) who also used SNP genotyping for the genetic diversity and detected the gene diversity and PIC values of 0.215 and 0.177, respectively. On the other hand, most of the researches involving SSR markers for genetic diversity detected high gene diversity and PICs values (Maccaferriet al., 2003 and Moragueset al., 2007).

However, the relative lower genetic variation revealed by SNP markers is expected. This is because SNP markers are mainly bi-allelic; and therefore, the gene diversity and PICs cannot exceed 0.5 while the multi-allelic markers such as SSR can approach the maximum of 1. Similarly, Chen et al. (2009) and Todorovska /et al. (2015) observed the overall genetic variation of 19.16% and 23.2% respectively across 47 SSRs and SNPs loci in 216 hybrids and elite breeding lines of tomato originating from four breeding centres in China.

The number of alleles per locus in this study was 2 alleles, although this value was expected due to the bi-allelic nature of the SNPs markers. Benoret al. (2008), reported 4.3 allele per locus and PIC value of 0.31 in tomato varieties. He et al. (2003) identified 2.7 alleles per locus on average and PIC value of 0.37 in the study of relationships among 17 varieties and two parental lines of tomato with 60 SSR markers. Limited allelic variation was also observed in a study of tomato populations consisting of a total of 216 genotypes from four breeding centres in China using 12 SSRs and 35 SNPs markers.

The present study revealed the genetic diversity within the F_3tomato genotypes. The relative high polymorphism (61.7%) recorded in this study for the fruit size characters was due to the occurrence of the null allele's segregation. The genetic similarity estimated in this study according to SNPs data was scaled up to 100%, thus suggesting the potential of SNPs markers in discriminating among tomato genotypes of close or distant genetic background.

Furthermore, it was reported that solanaceaus plants have a low frequency of polymorphism among cultivars (Nunomeet al., 2003 and Stagelet al., 2008). It was also documented that cultivated tomatoes are highly monophorphic at the molecular level, although they are phenotypically very diverse (Labate and Roberts, 2002).

Population structure

A prerequisite for the association studies is a good estimation of the true population structure. The result on the population structure was highly similar to the neighbour-joining dendrogram and fruit characteristics. Both neighbour-joining dendrogram and the population structure segmented the tomato genotypes into three main groups. The result validates the findings of Ruggieri et al. (2014) who also segmented tomato genotypes into three major groups by using both joining dendrogram and the population structure. The number of sub-populations obtained in this study was also similar to the number of clusters observed by Mazzucatoet al. (2007) in 61 accessions of the cultivated tomato. On the other hand, Ranc*et al*. (2012) detected only two sub-populations for the genetic structure of 90 tomato accessions using 20 SSR markers.

Materials and Methods

Plant materials and population development

The experimental materials comprised advanced hybrids raised from inter-specific crosses between cultivated tomatoes (Roma VF and Tropica) and the wild tomato relative, *Solanum pimpinefollium* (W x R, R xW and W x T). The advanced hybrids were crossed with a large fruited inbred tomato variety Supersteak (S) imported from the United States of America (USA), Beef (Florida) (BF) and Plumb (Rio grande) (PR) varieties in a modified three - way cross to produce F_1 hybrids (Figure 1). The advanced hybrids served as the pollen parent while the Supersteak, Beef and Plumb were the seed parents. The F_1 hybrids were selfed to produce the F_2 populations as a segregating population.

The experiments were carried out in the Department of Crop Science screenhouse, University of Nigeria, Nsukka, located in the derived savannah zone (Latitude 0.6^{o}52N, longitude 07^{o}24E with an altitude of 447.26 m above sea level) in 2013 and 2014. The seeds were raised in nursery boxes filled with sterilized soil, well cured poultry manure and river sand mixed at a ratio of 3:2:1 by volume. The seedlings were transplanted into polythene bags arranged in the screen house four weeks after planting.

DNA extraction

Leaf tissue was used to extract total genomic DNA from parents (advanced interspecific hybrid and supersteak) and the 94 F_2 populations arising from the crosses. The extraction of DNA followed the modified mini preparation protocol described by Doyle and Doyle (1990) with minor revisions. The extraction was done in the Department of Bioscience, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Single nucleotides polymorphism (SNPs) markers

Tomato genotypes were genotyped with 45 SNP markers (Supplementary 2). The SNPs markers were downloaded from the Tomato SNPs Database (SolCAP tomato collection) (Supplementary 1). SNPs markers selection and assay design

were performed according to the procedures of Chao et al. (2010). The SNPs were selected mainly from chromosomes that are related with fruit size and shape (chromosomes 2, 3 and 11). A total of 250ng of genomic DNA per genotype was used for the Illumina SNP genotyping at the Inqaba Biotech, Pretoria, South Africa using the Sequenom Mass Array Iplex Platform following the manufacturer's protocol (Gabriel et al., 2009).

Data analysis

The floral and fruit size and shape related traits were subjected to correlation analysis using the computer statistical software package, SPSS version 20. Path coefficient analyses were carried out to show direct and indirect effects (magnitude and significance) of the floral and fruit traits on the fruit size using the SASS statistical package. QTL association mapping was done using Trait Analysis by Association, Evolution and Linkage (TASSEL 3.0 version software). The population structure was estimated with the model-based (Bayesian) cluster software, STRUCTURE 2.33 version.

Conclusion

The genetic studies of the modified three ways cross tomato hybrids are necessary for the stable selection of marketable tomato fruit size. The SNP based analysis shows that a high level of genetic diversity exists among the modified three-way cross hybrids and population structure analysis reveals the occurrence of three different gene pools of F_2 tomato hybrids among the examined populations.The Single Nucleotides Polymophisms (SNPs) markers analysis implicated mean fruit weight, number of locules per fruit, fruit length, fruit diameter and fruit shape index in chromosome 2 and 11 as important determinants of fruit size. These traits were very evident in the determination of the fruit size and yield in S x (W x R), which is the most promising three- way cross.

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Data availability statements

The data that support the findings of this study are available from the corresponding author, Nnungu, Stephen. Issa, upon reasonable request.

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