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Photoperiod control of yield and sink capacity in Bambara groundnut (*Vigna subterranea*) genotypes

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Abstract

In Bambara groundnut [*Vigna subterranea* (L) Verdc], long photoperiods can cause a decline in pod and seed number. While negative photoperiod effects on pod yield have been reported, positive effects and the existence of genotypes less sensitive for photoperiod have not been reported. Ten geographically diverse genotypes were evaluated over 3 years under short and long photoperiod for yield-related traits (pod number, pod weight, seed number, seed weight, and 100 seed weight), pod growth habit (geocarpic or ageocarpic pods), peduncle elongation and soil penetration, and pod set/abortion. Anatomical sections of gynophores and embryonic pod development of a highly photoperiod sensitive genotype "Ankpa-4" from Nigeria were examined. A strong photoperiodic effect on yield component traits (pod number, pod weight, seed number, seed weight, and 100 seed weight) was observed and with considerable variation between genotypes. Three classes of photoperiod effect on pod yield were identified, (a) qualitative short-day types; (b) quantitative short-day types; and (c) quantitative long-day types. In long photoperiods, above-ground vegetative biomass and the length of lateral branches in some genotypes increased by at least twofold. Morphological and anatomical characterization of gynophores and developing pods of the most sensitive line shows continuous geocarpic growth, but with healthy embryonic pods failing to enlarge after soil penetration. Results from the yield patterns of the three phenotypic classes confirm that pods and seeds are high priority sinks, and long photoperiod alters the balance in assimilate distribution between competing yield forming processes and vegetative sinks. By exploiting the genetic variation characterized here for photoperiod requirement for pod filling, the geographical range of this crop could be extended beyond current growing regions. In the near future, we anticipate that Bambara groundnut will become a significant contributor to global food, nutritional, and environmental security once these photoperiod issues are resolved.

KEYWORDS

development, genetic variation, photoperiod effect, pod growth habit, pod yield, quantitative

1 | INTRODUCTION

An increasing worldwide demand for more nutritious food in the face of a rapidly changing climate has placed new emphasis on breeding future crops with higher yields, improved tolerance to abiotic stresses and, critically, better dietary nutritional content. Bambara groundnut [*Vigna subterranea* (L) Verdc] is a native African legume, able to grow on marginal lands and provides nutrition and a source of local income for smallholder farmers in sub-Saharan Africa. In addition to the high nutritional values of the seed (containing reasonable levels of protein (19%–21%), carbohydrate (57%–63%), and lipid (6%–8%) (Brough & Azam-Ali, 1992; Yusuf, Ayedun, & Sanni, 2008), the crop fixes nitrogen and is one of the more drought-tolerant crops (Collinson, Clawson, Azam-Ali, & Black, 1997; Jørgensen et al., 2010; Mabhaudhi & Modi, 2013; Vurayai, Emongor, & Moseki, 2011). Thus, Bambara groundnut is a potential future crop that could lend greater resilience to agricultural production under a rapidly changing climate. However, farmers have continued to cultivate landraces (a mixture of genotypes) that have been derived by selection and domestication from wild relatives (Anchirinah, Bennet-Lartey, & Yiridoe, 2001; Massawe, Mwale, Azam-Ali, & Roberts, 2005; Ntundu, Shillah, Marandu, & Christiansen, 2006). Seed yields can be low and/or unstable due to the lack of improvement by controlled cross-breeding. Variation in final yield has also been attributed to different planting dates (Karikari, Sebolai, & Munthali, 1996; Karikari et al., 1997; Linnemann & Craufurd, 1994), and low/unstable yields are more pronounced when landraces from equatorial regions are transferred and grown under long-day conditions away from the equator (Linnemann, 1994; Linnemann & Azam, 1993; Nishitani, Muraki, & Inoue, 1988; Sesay, Magagula, & Mansuetus, 2008).

The daylength at the equator (zero latitude) is reasonably stable, but toward the earth's poles the lengths of day and night change to become increasingly unequal. Changes in daily photoperiod (daylength) are one of the most important environmental stimuli influencing reproductive development in many crop species, due to its potential influence in determining the timing of the switch from vegetative to reproductive growth (Garner & Allard, 1920; Thomas & Vince-Prue, 1997). In Bambara groundnut in many landraces, photoperiod is also important for the successful set and filling of pods. Pod and seed number and seed grain weight both have strong effects on plant yield (Harris & Azam-Ali, 1993; Linnemann, 1991; Linnemann, Westphal, & Wessel, 1995). Bambara groundnut originated around the equator, between the borders of northern Nigeria and Cameroon and previous investigations have revealed that the crop requires around 12 hr of daylength for maximum pod and seed set (Harris & Azam-Ali, 1993; Linnemann et al., 1995). Depending on the latitudes and the environments in which it is grown,

Bambara groundnut is classified as a facultative short-day crop for pod set (Brink, 1999). White and Laing (1989) confirmed that common bean germplasm that originated from equatorial latitudes is relatively photoperiod sensitive, compared with germplasms from higher latitudes, indicated by delayed flowering and pod maturity when grown outside the center of origin. While the onset of flowering is rarely affected by long photoperiods, a nonpermissive photoperiod (>12 hr) can completely abolish pod set in some landraces, such as Ankpa4 and could lead to total crop yield "failure" (Brink, 1997; Harris & Azam-Ali, 1993; Jørgensen et al., 2009; Linnemann, 1991; Linnemann et al., 1995). However, many landraces are grown across a wide latitudinal distribution, ranging from landraces that are capable of producing pod yields only in equatorial latitudes (such as "Ankpa-4" from Nigeria; 12 hr daylength) to ones that produce variable yields across different planting times in the main growing season (Heller, Begemann, & Mushonga, 1997). The sensitivity of Bambara groundnut to long photoperiods often encountered when grown further away from the equator is a major potential constraint to the development of stable and yield predictable genotypes (Jørgensen et al., 2009; Linnemann, 1994; Linnemann & Azam, 1993; Nishitani et al., 1988). For smallholder farmers in various parts of Africa, where planting time is often determined by the timing of the rains, matching photoperiod requirement to actual daylength is likely to be one of the key aspects of improving crop productivity and adaptation. Therefore, an understanding of the underlying mechanisms behind photoperiod responses would facilitate the development of cultivars of this drought-tolerant species with wider geographical adaptation, and potentially enhance the successful exchange of germplasm between different latitudes. For example, a suitability mapping exercise in 2003 suggested that one of the most productive regions for growing Bambara groundnut would be in the countries surrounding the Mediterranean Basin (Azam-Ali, Aguilar-Manjarrez, & Bannayan-Avval, 2001), but at the moment many landraces may not produce pods. In the near future, it is anticipated that Bambara groundnut will become a significant contributor to global food, nutritional, and environmental security if photoperiod issues can be resolved.

Bambara groundnut is an indeterminate annual crop, with a growth habit that is typical of a prostrate and creeping plant at ground level with pods on the soil surface (ageocarpic) or below ground (geocarpic) depending on the length of an elongated peduncle (gynophore) after fertilization. After the initiation of pod enlargement on the soil surface or below ground, peduncle elongation ends and all the available carbohydrate supplies are channeled toward pod growth and seed filling. Lateral branch elongation and leaf growth may continue during pod set and there is sequential production of new flowers and pods, resulting in differences in pod ages at final harvest. However, all the pods can

continue to maturity when the plant enters senescence and seeds formed by the first flowers at the basal position of the stems tend to be larger in size than seeds developed from later formed flowers at distal positions (Doku & Karikari, 1970). Indeterminacy in grain legumes can be modified if growth and development are influenced by manipulation of the photoperiod (Han et al., 2006; Kantolic & Slafer, 2001; Nico, Miralles, & Kantolic, 2015). In long-day legume species such as peas (*Pisum sativum* L.), vegetative growth continued in short days, despite pod production and the accompanying nutrient demands by the pods (Gianfagna & Dvies, 1981; Murfet, 1977). This observation led to the conclusion that fruit is stronger sinks for assimilates under extended photoperiod conditions, and a high vegetative sink strength in short days may maintain assimilate supply toward shoot growth, delaying senescence (Kelly & Davies, 1988). Another example can be found in soybean [*Glycin max* (L) Merr.], a short-day legume. Exposure to long photoperiods has been shown to promote vegetative growth (Caffaro, Martignone, Torres, & Nakayama, 1988; Fleming, Ellis, John, Summerfield, & Roberts, 1997; Raper & Thomas, 1978), decrease dry matter partitioning toward pods and seeds (Ellis, Asumadu, Qi, & Summerfield, 2000), reduce seed growth rates (Morandi, Schussler, & Brenner, 1990), extend the reproductive period and increase seed numbers (Kantolic, Peralta, & Slafer, 2013; Kantolic & Slafer, 2007; Nico et al., 2015). For Bambara groundnut, photoperiod regulation of growth and development in the landrace selections "Ankpa-4" from Nigeria and "Tiga Nicuru" from Mali has been investigated and results showed a lack of pod set for "Ankpa-4" and a reduction in pod number for "Tiga Nicuru," mostly attributed to long photoperiod conditions (Linnemann, 1993; Linnemann et al., 1995). Further evidence from photo-thermal studies on selected Bambara groundnut landraces examined by Brink (1997) confirmed the partitioning of assimilates in favor of developing vegetative structures and a greater above-ground vegetative biomass at final harvest (183 days) under long photoperiods (Brink, 1998), suggesting that the developing pods in these landraces are weaker sinks for assimilate than the elongating lateral branches and new leaves (initially acting as vegetative sinks).

On the basis of the above observations, indeterminate legume species under varied photoperiod conditions are capable of producing an array of sinks (nonphotosynthetic organs), comprising vegetative sink tissues (stems, the elongating lateral branches, new leaves, and gynophores) as well as reproductive sink tissues (flowers, pods, and seeds), after a short initial phase of only vegetative growth. The developing pods compete with each other and with the vegetative organs for the assimilates available, implying that assimilate partitioning among sinks seems to be highly dependent on sink availability and the ability of the sink to demand assimilates (Ho, 1992; Marcelis, 1996). Despite the characteristic

differences in photoperiod response between long-day species, such as pea, and short-day species, such as soybean, photoperiod is also a primary regulator of growth and development in common bean (Wallace & Yan, 1998; Wallace, Yourstone, Masaya, & Zobel, 1993; Wallace, Zobel, & Yourstone, 1993). These studies further demonstrate that a higher strength vegetative sink in response to photoperiod leads to the allocation of more assimilates in favor of vegetative organs, causing a delay or inhibition of pod set. In contrast, if flowering time is daylength-insensitive, a photoperiod-induced increase in flower numbers and embryonic-pod formation might enhance the ability of developing reproductive organs to compete favorably for assimilates, thereby contributing to increased pod and seed number at final harvest. Knowledge accumulated on the photoperiod and genetic control of developmental transitions in model legume species and particularly carbon partitioning in peas and its relationship to apical senescence and also the reproductive period in soybean, have provided important insights into the general physiological basis and genetic control of these developmental processes (Cheng, Wang, & Zhang, 2011; Kelly & Davies, 1988). An indeterminate growth habit over a wide range of photoperiod conditions gives most Bambara groundnut accessions a high degree of plasticity in their ability to modify above-ground vegetative dry matter, as well as pod and seed yield, and hence, crop productivity.

The mechanisms responsible for the failure of pod enlargement or a reduction in pod yield in Bambara groundnut caused by long photoperiods are not well established. Neither are there any reported photoperiod insensitive genotypes. In this work, we exploited local adaptation to different latitudes in diverse genotypes to assess Bambara groundnut yield determination in response to different photoperiods. The objective of this study was to investigate the photoperiod control of pod and seed number in Bambara groundnut with an emphasis on static long-day (LD) effects. Our hypothesis is that the increase in pod and seed number per plant is associated with a decline in individual seed weight due to the increased number of sinks and is causally linked to competition among simultaneously developing sinks (the elongating lateral branches and increased pod number). To test this hypothesis, we have measured the effects of the photoperiod treatment by comparing the above-ground vegetative dry matter (lateral branches and biomass) and major reproductive traits (pods and seed yield per plant) in Bambara groundnut for ten different genotypes from different collection origins worldwide. These were grown in different photoperiods in identical soil beds in a complete randomized design, leading to the conclusion that there are intrinsic differences in sink strength between genotypes and in different photoperiod regimes. We have also dissected the postfertilization elongated peduncles (equivalent to the peanut gynophore) and embryonic pods of the highly photoperiod sensitive genotype "Ankpa-4" from

Nigeria to understand the inhibition of pod development under long photoperiods.

2 | MATERIALS AND METHODS

2.1 | Experimental conditions, plant material, and treatments

The experiments were conducted in three climate-controlled glasshouses ("FutureCrop") located at the University of Nottingham Sutton Bonington campus, UK (latitude 52°50′N, 1°15′W). The original design and control system of the glasshouses are quite similar to the agronomy-style glasshouses described in Monteith et al. (1983), but with major improvements in technical specifications and alterations built by the CambridgeHOK Glasshouse Company (Brough, UK) in 2011. Each greenhouse compartment has a total cropping area of 50 m^2 divided into two lined soil pits, and each soil pit is a 25 m^2 plot containing a sandy loam soil. In all glasshouses, daylength was controlled automatically from sowing to final harvest at 160 days after sowing (DAS) using blackout screens. Day and night temperatures were set at 28°C and 23°C.

Plant materials consisted of ten Bambara groundnut genotypes derived from landraces originating from different locations in Africa, with contrasting expected daylength at the time of flowering in the main growing season within country. Details of these plant materials, country of origin, and geographical proximity to the equator are listed in Table 1. These landraces are adapted to tropical and subtropical conditions in the major Bambara groundnut growing regions of

sub-Saharan Africa and Indonesia. As a self-pollinating crop, genotypes used were selected and seed obtained from single plants of each landrace that had been planted in previous years. Bambara groundnut is strongly inbreeding (Molosiwa, Aliyu, & Stadler, 2015; average 2% heterozygosity), so that the majority of genotype lines are expected to be less than 5% heterozygous, which is equivalent to an F$_6$ generation of inbred lines.

Experiments on the ten parental lines were conducted on plants grown as crop stands, 25 cm × 25 cm within and between plant rows during the summer seasons of 2012, 2013, and 2014. The experiment was laid out in a split-plot design with photoperiod [12-hr (0600–1800) and 16-hr (0600–2200) as the main factor and genotypes as the split-factor (Ankpa4, Dip C, DodR, Getso, Gresik, IITA-686, Lun T, S19-3, Tiga Nicuru, and Uniswa Red) arranged in a completely randomized design (CRD), with eight replications. Each photoperiod was assigned to a separate glasshouse; glasshouses were interchanged during the 3-years trials to reduce any error or bias and phenotypes were measured for four plants per genotype under each imposed photoperiod treatment. Light in each glasshouse was controlled by an automatic blackout screen. The photoperiod treatment was rotated between the three glasshouses over 3 years, so that each of the glasshouses received the 12-hr/16-hr treatment at least once during the 3 years of phenotypic characterization. While a photoperiod of 12 hr/d was considered permissive, photoperiods of 16 hr/d were considered to be extreme for pod development in a short-day plant such as Bambara groundnut. Natural lighting was complemented by High Pressure Sodium lamps, (HortiluxSchreder, HPS600W/400V) in

TABLE 1 Background of genotypes used in the experiment together with details of origin, Growth, and podding habits

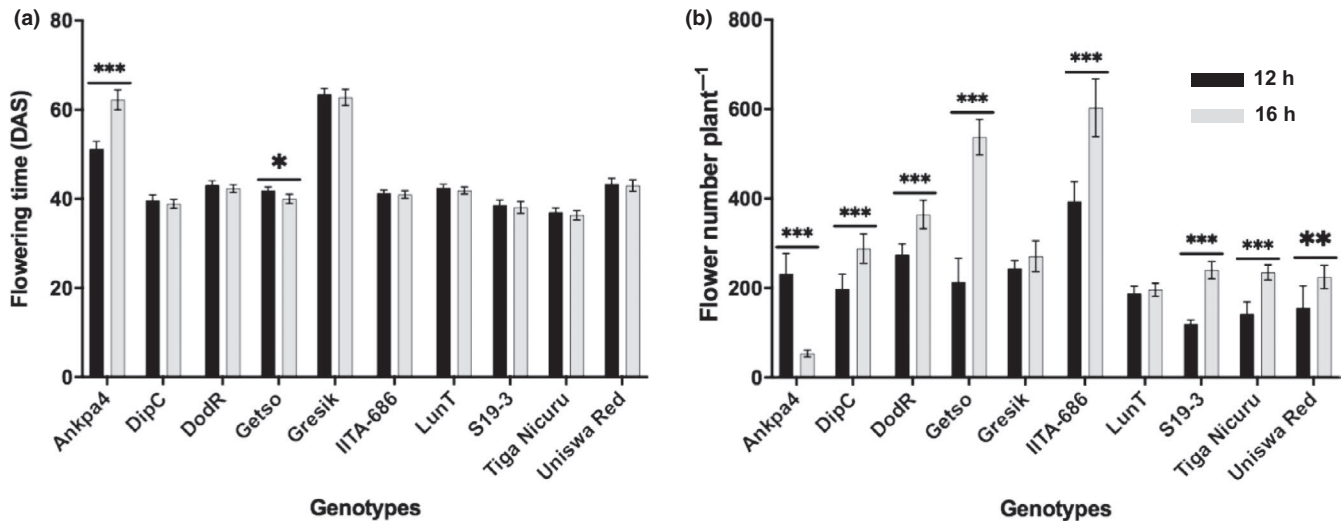| Genotype	Growth habit	Podding habit	Geographical origin	Location
Ankpa 4	Spreading	Buried	7.24°N, 7.38°E	Ankpa, Nigeria
Dip C	Bunched	Pod on soil	24.38°S, 25.55°E	Diphiri, Botswana
DodR	Spreading	Buried	5.54°S, 35.57°E	Dodoma, Tanzania
Getso	Spreading	Buried	12°N, 8.31°E	Kano, Nigeria
Gresik	Spreading	Pod on soil	7.09°S, 112.39°E	Gresik, Indonesia
IITA-686	Spreading	Pod on soil	6.10°S, 35.46°E	Tanzania
Lun T	Spreading	Buried	8.29°N, 13.14°W	Lungi, Sierra Leone
S19-3	Semi-bunched	Pod on soil	22.33°S, 17.04°E	Namibia
Tiga nicuru	Semi-bunched	Buried	13.14°N, 7.59°W	Mali
Uniswa Red	Bunched	Pod on soil	26.28°S, 31.22°E	Manzini, Swaziland

FIGURE 1 The effect of photoperiod on flowering time (a), and flower number (b) in 10 Bambara groundnut genotypes from (a) 2012–2014; (b) 2012–2013. All data are mean values of three growing seasons for (a), 2012 through 2014; and two growing seasons for (b). The vertical bars represent the mean $\pm$ SD (***, significant with $p < .001$; **, significant with $p < .01$; *, significant with $p < .05$)

all greenhouses from May until the end of the experiment (October/November). PAR of the supplementary lighting was 212 ± 9 µmol photons m^{-2} s^{-1} at 2 m below the lamps. The lamps were turned on automatically whenever the natural light level dropped below 212 µmol/m^2 s^{-1} and turned off when it exceeded 212 µmol/m^2 s^{-1}.

Seeds of the 10 genotype lines of Bambara groundnut were sown (2–3 cm planting depth) in 96-cell seedling trays containing modular seed compost (F2 + S). At 7 days after emergence, 8 seedlings per genotypic line for each of the twenty treatment combinations were transplanted directly into the greenhouse soil in glasshouses on the 23rd of May 2012 and 25th of May 2013 and 2014. These experiments were each carried out for a period of 5 months and plants harvested on the 30th of October 2012 and 1st of November 2013/2015, respectively. Four plants in between border guard rows per genotype were used in the final analysis (4 replicates). Irrigation was supplied by trickle-tape for 10 min morning and 10 min evening throughout the experiment period. This accounted for a flow rate of 4 L/day, supplied to 40 plants in 2 rows (20 plants each). The two different photoperiod treatments were imposed from sowing in each of the glasshouses, and data were collected according to standard IPGRI descriptors (IPGRI, 2000) for *days to emergence*, *days to first flower opening* (anthesis), and the yield-related traits (*number of pods plant*$^{-1}$, *number of seeds plant*$^{-1}$, and *100-seed weight plant*$^{-1}$). Measurements were nondestructive, and direct observations at the beginning of pod set were not possible because some of the selections included in the experiment mostly buried their pods (e.g., Ankpa4, Tiga Nicuru, LunT, and Getso). Maturity was established for plants grown under 12-hr as a standard, when leaves began to senesce and

pods began to dry-out. Seeds were dried to 12% moisture at 37°C for 2 weeks and above-ground dry matter at 85°C (Oven dry weight) for 48 hr. The yield component traits were determined at final harvest and after processing of dried pods.

2.2 | Anatomical sectioning

Newly open flowers of Ankpa-4 under 12 hr and 16 hr photoperiod conditions were identified daily (10:00–14:00) by marking the target node with acrylic paint at anthesis, so that pods and gynophores of known age could be collected. These embryonic pods under the 16 hr photoperiod (1–2 mm wide and 3–4 mm long) were collected at 3, 12, 29, and 45 days after anthesis, and gynophores (5–10 mm long) were immediately fixed overnight in a 4°C fixation solution containing 4% paraformaldehyde in 1xPBS (pH 7.2), 0.1% Triton X-100, and 0.1% Tween 20. Following fixation, samples were processed through a 30-min vacuum treatment and were left overnight in a vertical rotor at 4C. The following day, samples were washed for 2× 30 min in 1× PBS at 4°C at room temperature. Tissues were then dehydrated in a graded ethanol series and then embedded in Spurr's low-viscosity resin (TAAB, S024/D). Ultra-thin (500–800 nm thick) sections were cut with glass knives on a Leica Ultramicrotome EMUC6 (Leica) and dried onto coated microscope slides (Fisher Scientific Co.). Three blocks were sectioned for each time point, and a minimum of 80 sections were collected for each block. Sections were stained with 0.25% toluidine blue O in 1% Sodium Borate and observed with a Leica DM5000B microscope (Leica). Digital microscopic images were taken using a Leica DFC420C camera (Leica).

2.3 | Statistical analyses

Data were subjected to analysis of variance (ANOVA) using the GenStat software 16th Edition (VSN International Ltd) and GraphPad Prism version 8.03 for Windows (GraphPad software, Sandiego, California USA) to observe the difference between genotypes and photoperiod treatments, and to test for a genotype x photoperiod interaction. Means were compared for significant differences using LSD at a significant level of 5%.

3 | RESULTS

3.1 | Effects of photoperiod on flowering

Under the experimental conditions in the tropical greenhouse, the onset of flowering, measured in days after sowing (DAS), was significantly different between genotypes ($p < .001$) but not affected overall by photoperiod (Figure 1a, Table 2). However, flowering time across the two photoperiod treatments did interact significantly with genotypes ($p < .001$). The largest interaction between photoperiod and genotype was observed in the genotype "Ankpa 4," which flowered earlier under 12 hr (51.25 ± 1.9 DAS in 2012 and 51.5 ± 1.3 DAS in 2013) compared to its flowering time under 16 hr (60.75 ± 2.2–63.25 ± 2.5 DAS). The delay in the flowering time for Ankpa4 when grown under 16 hr was consistent with Linnemann (1993). In terms of the number of flowers produced per plant for each of the 2012 and 2013 seasons, there were significant differences across genotypes, photoperiod regime, and an interaction between genotypes and photoperiod (Figure 1b, Table 2). Flower numbers for Dip C, DodR, Getso, Gresik, IITA-686, Lun T, S19-3, Tiga Nicuru, and Uniswa Red were higher in 16 hr compared with 12. Only for Ankpa4 were lower numbers of flowers per plant observed in 16 hr compared to 12 hr. There was a significant interaction between genotype and photoperiod regime on total flower number per plant for all genotypic lines.

3.2 | Effects of photoperiod treatment on yield components

The different photoperiod treatments significantly affected yield components (pod weight, seed number, seed weight, 100 seed weight per plant, and harvest index) of Bambara groundnut (Figures 2–4; Table 2). In the Ankpa-4 genotype, there was no pod enlargement in 16 hr at final harvest. This genotype was classed as "qualitative short-day." The number of matured pods set, seed number, and total seed yield per plant of TN, Getso, LunT, Gresik, and DodR, was significantly higher in the shorter photoperiod than in the longer photoperiod treatment. Under the long photoperiod treatment, this set of genotypes developed fewer pods and seeds per plant and had lower total seed weight. (Figures 2 and 3). There was no significant delay in the flowering time for this group of genotypes, and growth and embryonic pod set continued under the long photoperiod even when the same genotypes under the short photoperiod treatment had begun to senesce. The yield per plant and total seed weight were significantly higher in the 12 hr than in the 16 hr photoperiod treatment. This group was classed as "quantitative short-day." Three genotypes with origins from further away from the equator Dip C (24.38°S), S19-3 (22.33°S), Uniswa Red (26.28°S) and one from around the equator, IITA-686 (6.10°S), were indeterminate in vegetative growth and development under the longer photoperiod and, by contrast, the yield per plant was higher in the long photoperiod treatment than in short photoperiod treatment, but with smaller individual seed weight and later maturity time, and these were classified as "quantitative long-day" types. The 100 seed weight and harvest index of most Bambara groundnut genotypes exposed to the longer photoperiod were significantly lower than that of plants exposed to the shorter photoperiod (Figure 4). The results of the present study suggest that the shorter photoperiod promoted pod and seed formation with dry matter partitioning toward reproductive sinks at pod set (Figure 4b).

TABLE 2 Summary of *F* probabilities from analysis of variance for photoperiod (P), Genotypes (G), photoperiod × genotype (P × G) interaction on flowering time (FT), flower number (FN), pod number (PN), pod weight (PW), seed number (SN), seed weight (SW), 100 seed weight (100SW), harvest index (HI), lateral branch length (LBL), and vegetative biomass (VB) from 2012 to 2014

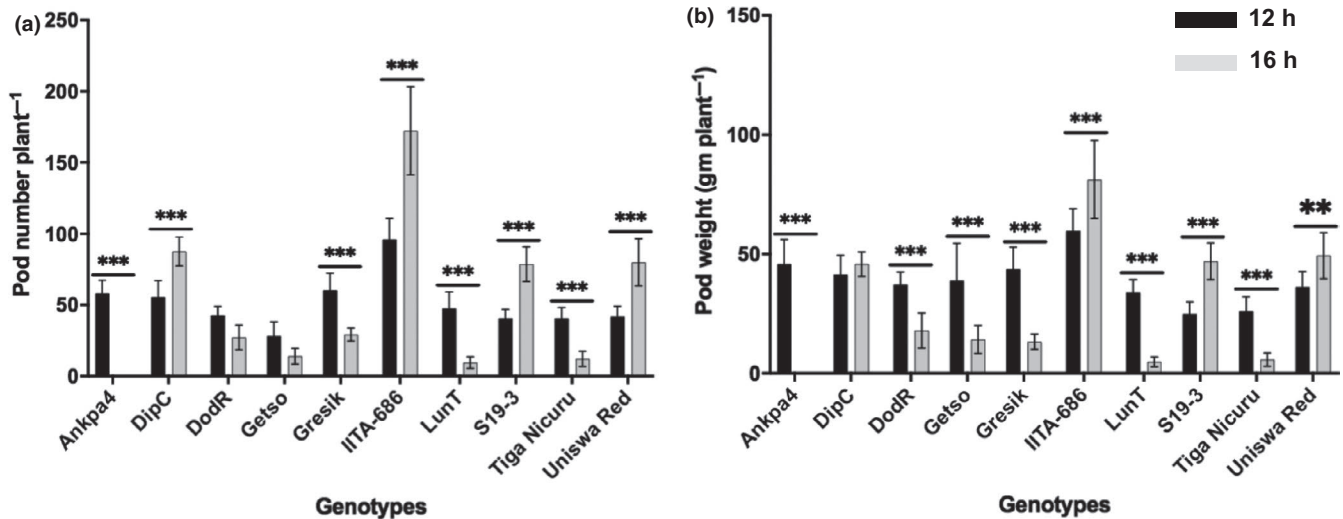| Sources of variation	df	*F* probability × variable									
		FT	FN	PN	PW	SN	SW	100SW	HI	LBL	VB
Years	2	0.203	0.005	0.06	0.081	0.195	0.32	0.749	0.129	<0.001	<0.001
P	1	0.128	<0.001	0.922	0.003	0.009	0.003	<0.001	<0.001	<0.001	<0.001
G	9	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P × G	9	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	120	2.8	11.6	19.2	19.5	17.7	20.8	11.5	9.4	13.1	14.2

FIGURE 2 The effect of photoperiod on pod number (a), and pod weight (b) in 10 Bambara groundnut genotypes from 2012 to 2014. All data are mean values of three growing seasons, 2012 through 2014. The vertical bars represent the mean $\pm$ *SD* (***, significant with $p < .001$; **, significant with $p < .01$; *, significant with $p < .05$)
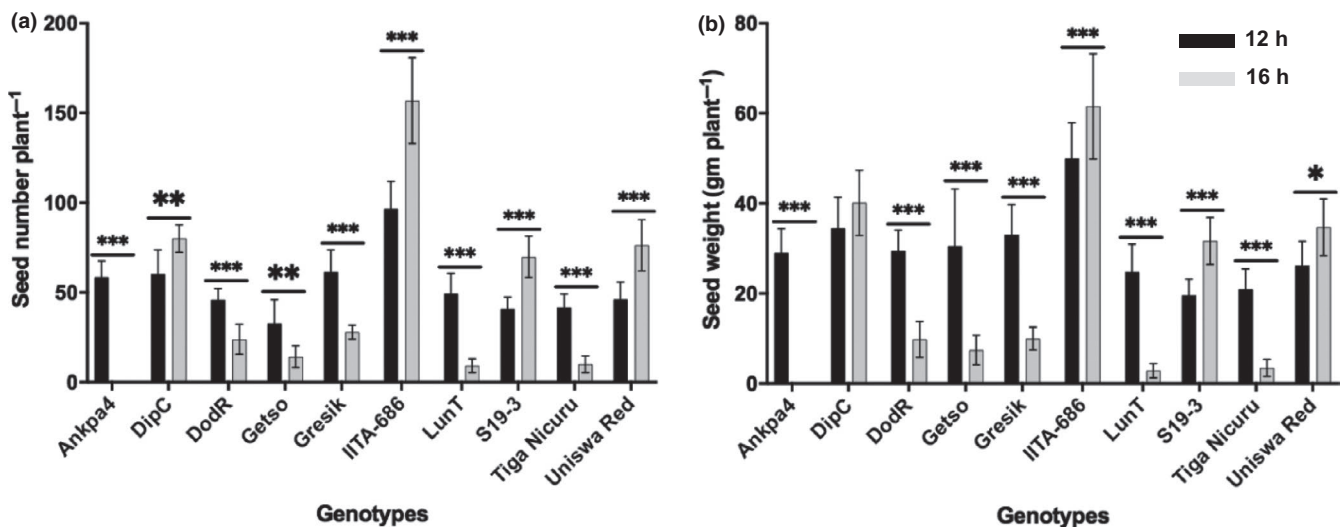

FIGURE 3 The effect of photoperiod on seed number (a), and seed weight (b) in 10 Bambara groundnut genotypes from 2012 to 2014. All data are mean values of three growing seasons, 2012 through 2014. The vertical bars represent the mean $\pm$ *SD* (***, significant with $p < .001$; **, significant with $p < .01$; *, significant with $p < .05$)

3.3 | Effects of photoperiod on lateral branch and above-ground vegetative growth

Measured at harvest (160 DAS), long photoperiod had a strongly increasing effect on the length of lateral branches and total plant above-ground vegetative dry matter in all genotypes (Figure 5, Table 2). Both above-ground vegetative traits were significantly different across genotypes, photoperiod treatments and with an interaction between genotype and photoperiod present in all years ($p < .001$). Lengthening the photoperiod increased

the average length of lateral branches from 18.94 ± 12.6 (12 hr) to 43.13 ± 18 (16 hr) and average above-ground vegetative biomass from 44.99 ± 21.3 under 12 hr to 90.76 ± 34.3 under 16 hr (Figure 5). Under a photoperiod of 16 hr, genotypes with spreading growth habits and elongated internodes (Table 1) produced greater vegetative biomass than genotypes that were bunched or semi-bunched. The effects of the photoperiod treatment increased lateral branch length and total above-ground vegetative biomass. Overall, Ankpa4 produced the most above-ground biomass in both photoperiod treatments (Figure 5).
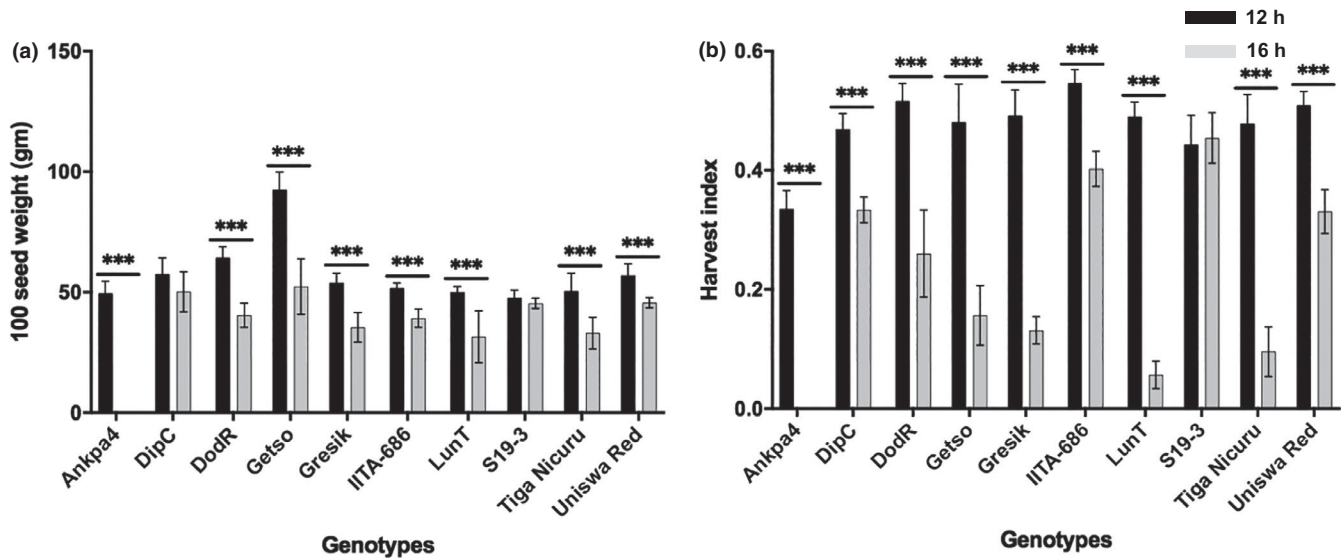
FIGURE 4 The effect of photoperiod 100 seed weight (a), and harvest index (b) in 10 Bambara groundnut genotypes from 2012 to 2014. All data are mean values of three growing seasons, 2012 through 2014. The vertical bars represent the mean ± SD (***, significant with $p < .001$; **, significant with $p < .01$; *, significant with $p < .05$)
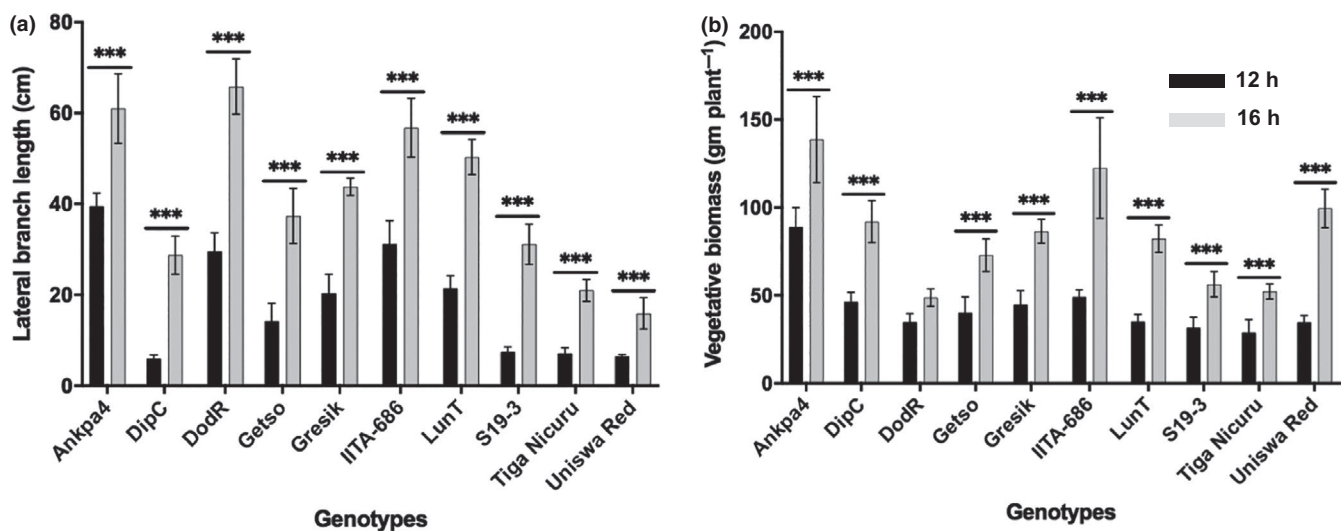

FIGURE 5 The effect of photoperiod lateral branch length (a), and above-ground vegetative biomass (b) in 10 Bambara groundnut genotypes from 2012 to 2014. All data are mean values of three growing seasons, 2012 through 2014. The vertical bars represent the mean ± SD (***, significant with $p < .001$; **, significant with $p < .01$; *, significant with $p < .05$)

3.4 | Effects of photoperiod on pod development in Ankpa-4

The cause of the failure in pod development and enlargement under the long photoperiod treatment of 16 hr, as observed consistently for 3 years was further investigated by comparing developing pods and gynophores formed under photoperiods of 12 hr and 16 hr. Fertilization was observed 24 hr after anthesis, the pedicel bends toward the peduncle, and embryonic pods were formed, irrespective of photoperiod (Figure 6), but none of the developing pods under the 16 hr photoperiod treatment had grown beyond the embryonic stage at harvesting.

After successful fertilization, the peduncle is transformed into a gynophore, which transports the embryonic pod into the soil in preparation for geocarpic pod formation. The gynophore of Bambara groundnut is an elongated peduncle, a product of fertilization, but with an internal structure of a typical dicotyledonous stem (Figure 7c). It was observed that the developing gynophores under 12 hr photoperiod were healthier than the gynophores growing under the 16 hr photoperiod; however, there was no difference in internal structures between the gynophores that penetrated the soil under both photoperiod conditions at 45–50 days after anthesis (Figure 8). Throughout the reproductive period, the embryonic pods of Ankpa-4 plants

FIGURE 6 Different stages of Ankpa4 pod development under 12 hr (a–c) and 16 hr (d–f) photoperiod: (a) day-1 of anthesis; (b) 3-days after fertilization; (c) fruit development under 12 hr photoperiod; (d) day-1 of anthesis; (e) 3-days after fertilization; (f) failure of fruit development under 16 hr photoperiod; (g) 3-days after fertilization, i = pedicel, ii = embryonic pod, iii = flower, (h) cross-section of embryonic pod; (i) longitudinal section of embryonic pod, showing the fertilized ovules in the middle. Scale bars: c and f = 5 mm, g = 3 mm, h and i = 0.1 mm

under the 16 hr photoperiod treatment never increased in size, although fertilized ovules were formed (Figure 6f). Under the 12 hr photoperiod, pod enlargement was observed, indicating that short photoperiod promoted pod set and, more importantly, pod filling and maturity in this genotype (Figure 6c), although some embryonic pods that set later and in positions further away from the base of the plant in all genotypes could not enlarge fully under the 12 hr photoperiod. This failure in complete pod expansion is not likely to be attributed to the photoperiod effect, but rather is likely to be due to higher demand for assimilates from the earlier formed pods and lack of developmental time to mature for late set pods.

4 | DISCUSSION

The results suggested that photoperiod does not significantly affect the flowering time of most genotypes. The only genotype that had a significant delay of 6–7 days to the

onset of flowering under the long photoperiod was Ankpa4 from Nigeria (7°N), and this selection has been previously reported to show daylength sensitivity for days to flowering (Brink, 1997, 1999; Linnemann, 1993, 1994). In addition to the delay in flower bud initiation for Ankpa4, we also observed an inhibition of the flowering process itself, whereby the number of flowers produced per plant was reduced from 217 ± 29.5 in the 12 hr treatment to 52 ± 4.25 in the 16 hr treatment. The photoperiod effect on flowering progress in this genotype line of Bambara groundnut appears to be similar to that in common bean genotypes, which are photoperiod insensitive for flower bud initiation, but photoperiod sensitive for continued development of flower buds. Wallace, Yourstone, et al. (1993) and Wallace, Zobel, et al. (1993) suggested the delay in flowering under long days is a result of slowly growing buds and/or abortion. After floral initiation, Patel and Hall (1990) showed that night temperatures enhanced by long photoperiods may suppress floral bud development, and Hall (1993) confirmed that a single
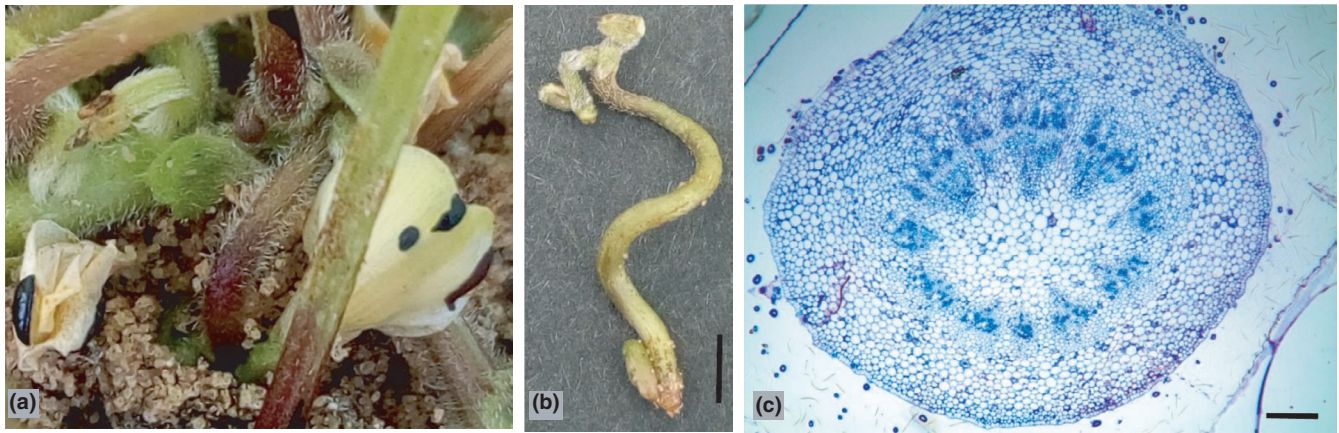
FIGURE 7 Bambara groundnut gynophore development: (a) after fertilization; (b) after soil penetration; (c) cross-section of a Bambara groundnut gynophore, showing its typical dicot stem anatomy. The pith is surrounded by a ring of vascular bundles, with the cortex and epidermis in the outermost region. Scale bars: b = 5 mm, c = 0.1 mm

recessive gene controlled such heat-induced floral bud suppression. In the case of Ankpa4, a possible explanation for the delay and flowering inhibition might be a combination of gene action and genotypic adaptation to equatorial latitudes. Compared to every other African selection included in this study, Ankpa4 also took the longest time to flower. This qualifies Ankpa4 both as a late flowering genotype, and as one that is highly affected by photoperiod. Gresik from Indonesia is another Bambara groundnut selection from around the equator (6°S) that inherently flowers late, but its flowering is not delayed or inhibited significantly by the photoperiod treatment.

A higher cumulative number of flowers per plant was produced by all landraces under the long photoperiod treatment (16 hr), with the exception of Ankpa4 (as above) compared to the short photoperiod treatment (12 hr) in the 2012 and 2013 growing seasons. This suggests that long photoperiod and possibly an effect of increased assimilate supply promotes flower bud initiation and flower number in most Bambara groundnut genotypes, especially when grown under ideal temperatures like those found in the tropics. The finding that photoperiod significantly affected the cumulative number of flowers per plant is in agreement with Brink (1999) who showed that the increased number of flowers per plant between the time of flowering and pod set ranged from 350% to 400%, as the photoperiod length increased (10.5 hr/day–14.5 hr/day). This was partly attributed to the longer interval between flowering and podding under longer photoperiods. However, Brink (1999) also observed that the number of flowers per day was influenced by photoperiod. In contrast, the cumulative number of flowers per plant was suppressed by long-day treatments in peanut (Bagnall & King, 1991a, 1991b; Bell, Bagnall, & Harch, 1991; Emery, Sherman, & Vickers, 1981; Flohr, Williams, & Lenz, 1990; Wynne, Emery, & Downs, 1973). At 60 –70 days from emergence, flower numbers for some peanut genotypes approximately

doubled in 12 hr days compared with plants in 16 hr days (Bagnall & King, 1991a).

In most of the Bambara groundnut selections included in this study, the response for pod set and yield production was quantitative rather than qualitative (Figure 9). This indicates a high level of diversity in photoperiod response for landraces collected from different geographical locations. Among the selections from around the equator, it was confirmed that Ankpa4 (7°N) was the only landrace that could not produce any matured pods when grown at a daylength of 16 hr. Five genotypes (DodR, LunT, Getso, Tiga Nicuru, and Gresik) from latitudes 7–14°N/S had clearly defined pod set that was quantitative for short days. For this class of genotype, there was an effective decline in pod and seed yield with increasing photoperiod (12-hr–16-hr). The yield per plant and total seed weight were significantly higher in the 12 hr than in the 16 hr photoperiod treatment, indicating the partitioning of assimilates toward the higher order sinks of pod and seed formation under 12 hr. In contrast, IITA-686 from Tanzania (6°S) and three other genotypes (DipC, S19-3, and Uniswa Red) from latitudes 22–26°S produced more pods under 16 hr than they produced under 12 hr of photoperiod, albeit with an extension of the flowering period and a later maturity date. From these results, it can be inferred that most of the Bambara groundnut selections included in this study were quantitative for pod set. These results support experiments with some of the same genotypes (Ankpa4 and Tiga Nicuru) in controlled environments (Jørgensen et al., 2009; Linnemann et al., 1995), and Tiga Nicuru is recognized as being a photoperiod less-sensitive type for pod set than Ankpa4. The identification of quantitative short-day types of Bambara groundnut for pod number and seed yield is similar to the photoperiod effects on peg and pod numbers revealed in peanut genotypes that are insensitive for photoperiod effects on flowering (Bagnall & King, 1991a; Bell et al., 1991; Flohr et al., 1990). However, the characterization of quantitative long-day types is a new observation for
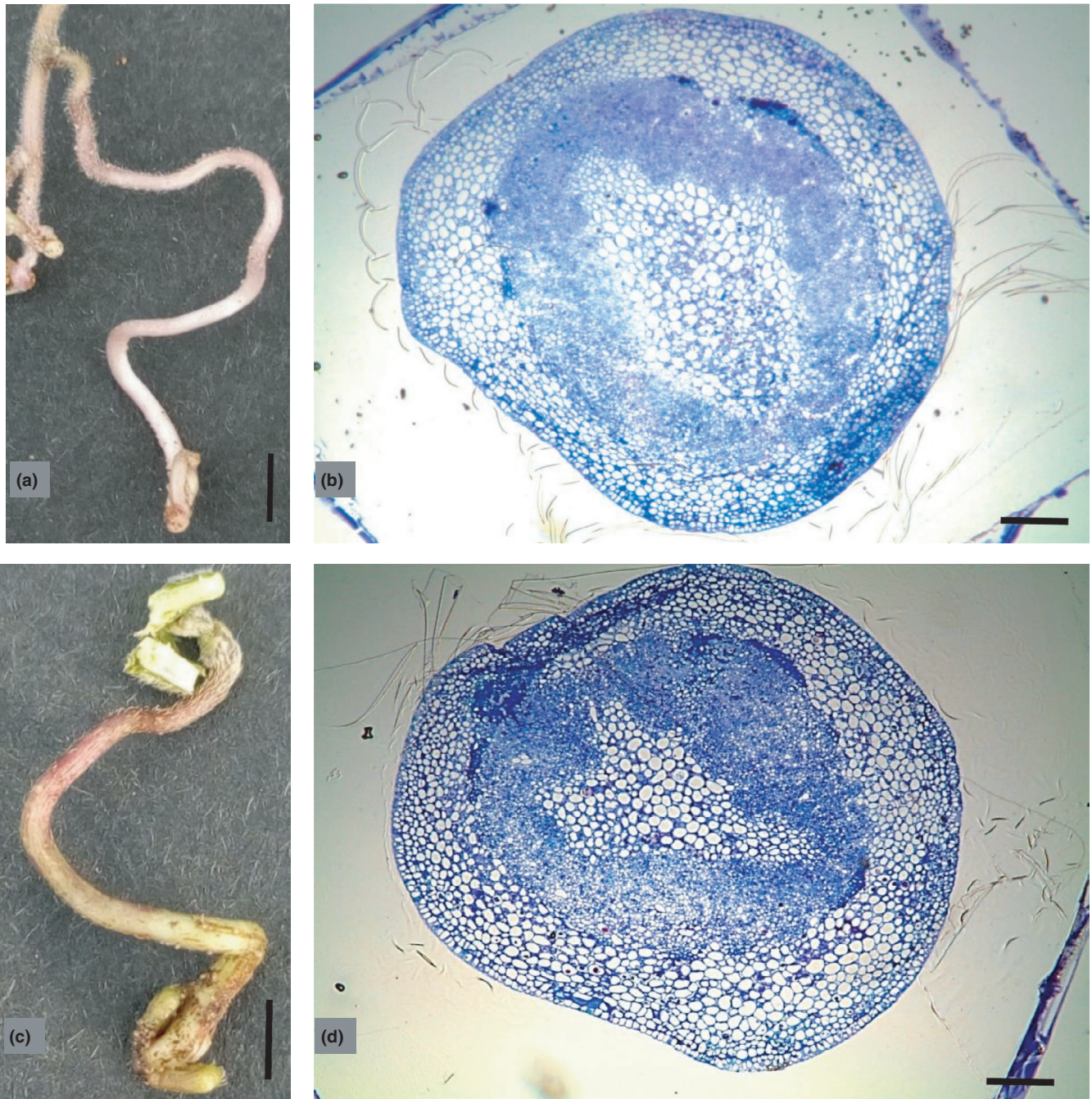
FIGURE 8 Ankpa-4 gynophores and cross-sections at 45–50 days after anthesis: (a, b) under 16 hr photoperiod; (c, d) under 12 hr photoperiod. The pith is surrounded by a ring of vascular bundles, with the cortex and epidermis in the outermost region. Scale bars: a and c = 5 mm, b and d = 0.1 mm

this species. Brink (1999) showed that the onset of podding was the most important event in the phenology of Bambara groundnut, because it coincides with a major shift in assimilate partitioning and the end of vegetative growth. However, in this study, we highlight the importance of relying on final harvest data in defining the overall daylength response of crop plants that exhibit insensitivity to photoperiod at flowering. Photoperiod influences Bambara groundnut fruit development but mainly has effects on critical yield determining traits (pod number, pod weight, seed number, seed weight, and 100

seed weight). Such continued sensitivity to daylength after pod set may help in establishing a better link between post-flowering responses to photoperiod and yield. It was not possible for us to quantify the rate of progress to pod set clearly, because a number of the landrace derived genotype lines set pods below ground (e.g., LunT, Ankpa4, Getso, Uniswa Red, DodR, and Tiga Nikuru). However, the harvest data obtained for all landraces at 150–160 days after sowing (DAS) for all treatments do provide information on pod number and seed yield in Bambara groundnut production.
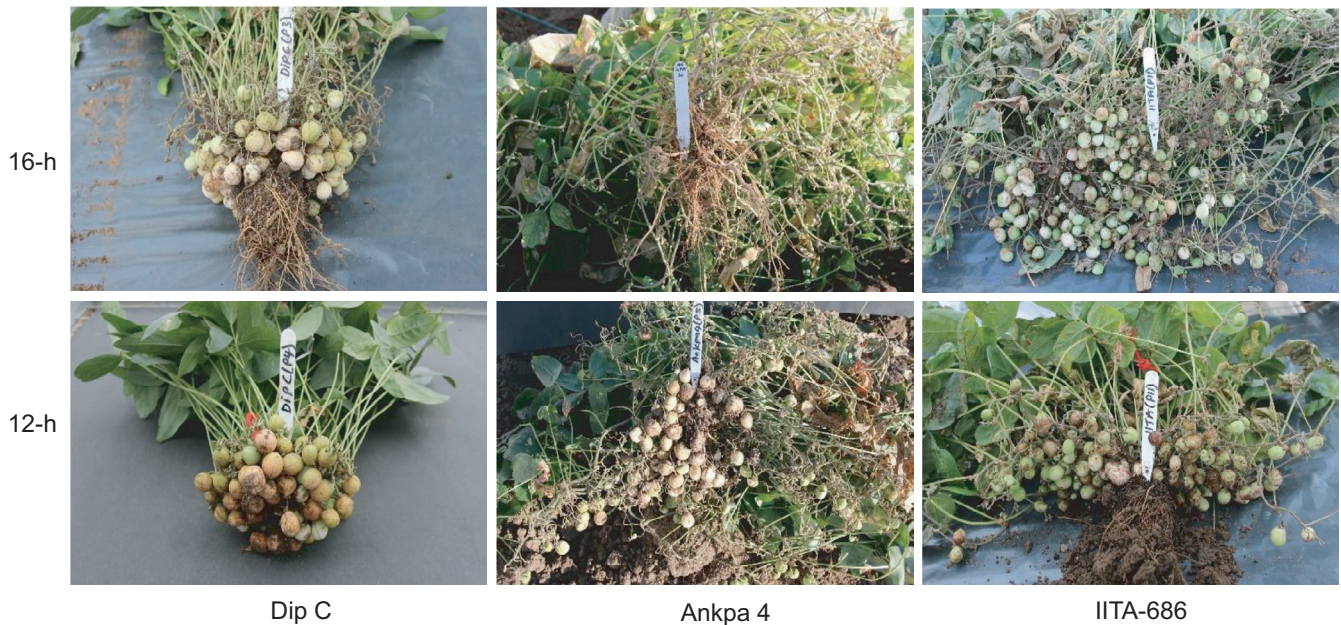
	Dip C	Ankpa 4	IITA-686
16-h			
12-h			

FIGURE 9 Typical variation in pod set of Bambara groundnut genotypes under different photoperiod treatment

One interesting response found in IITA-686 was that total pod number and total seed weight almost doubled under the 16 hr photoperiod compared with 12 hr. The development of pods in this selection continued, even as leaf growth, stem elongation, and increased flowering occurred. These results do not completely agree with those of Brink (1999), who concluded that pod set in Bambara groundnut during long photoperiods was accompanied by a major shift in assimilate partitioning and the end of vegetative growth. There is evidence in indeterminate soybean that long photoperiods extended the duration of the postflowering phases (Guiamet and Nakayama, 1984; Han et al., 2006; Kantolic & Slafer, 2001; Summerfield, Asumadu, Ellis, & Qi, 1998; Thomas & Raper, 1976), thereby leading to the formation of more pods and seed yield (Kantolic & Slafer, 2001, 2005, 2007) as has been seen here in the quantitative long-day types. This response in Bambara groundnut could be an important component of the mechanisms involved in the incremental changes in flower and pod numbers during longer photoperiods. Lengthening the photoperiod promotes indeterminacy in Bambara groundnut genotypes (IITA-686, Dip C, S19-3, and Uniswa Red), which are classified as quantitative for long photoperiod in terms of pod and seed production. From a productivity standpoint, these materials should be of interest to Bambara groundnut breeders who wish to improve yield and stability in different environmental conditions. The main disadvantage is the delay in these genotypes in reaching maturity. Interestingly, the genotype IITA-686 was collected from Tanzania (6°S), highlighting the problems of assuming that crop plants originating from equatorial latitudes are more (or less) photoperiod sensitive and therefore should perform worse than selections from nonequatorial latitudes in terms

of yield. Unlike Ankpa4 and other quantitative short-day genotypes that produce gynophores and geocarpic pods, the contribution of long photoperiod to the increased pod number and seed yields in the quantitative long-day genotypes could partly be attributed to higher fertility, inherent ability to produce more flowers and subsequent conversion into pods on the soil surface (in the absence of peduncle elongation after fertilization). Another possibility could be the lack or weak influence of photoperiod genes, allowing assimilates to be partitioned to potential pods or seeds, whereas strong photoperiod effects lead to partitioning to vegetative organs (Wallace, Yourstone, et al., 1993; Wallace, Zobel, et al., 1993). The flower production of Ankpa4 is relatively poor in 16 hr and the reproductive sink strength is presumably weak, which is evident by the total number of flowers and pods produced per plant in the longer photoperiod (Figure 1b). The negative effect of photoperiod on pod and seed yield in this genotype appears partly related to a delay in the onset of flowering, elongation of gynophores, and an indeterminate vegetative growth habit, which enabled the utilization of greater amounts of assimilates for vegetative sink formation under the long photoperiod treatment. The effects of the photoperiod treatment increased lateral branch length and total above-ground vegetative biomass in a species that is simultaneously growing vegetative organs, flowering, setting pods, and filling seed. Overall, Ankpa4 produced the highest amount of above-ground biomass in both photoperiod treatments, allocating more resources to vegetative growth, with little or no conversion of biomass into seed in 16 hr (Figure 4). Theories as to the cause of failure in pod set and seed production in lupin species (grain legumes in which only a small proportion of flowers normally form fruit) include hypotheses based on

competition for nutrients (Downes & Gladstones, 1984; Pate & Farrington, 1981) and those involving hormonal influences that promote the set of pods at some floret positions or inhibit pod set at other floret positions (Emery, Ma, & Atkins, 2000). In *Lupinus angostifolius, L. luteus,* and *L. mutabilis,* for example, lateral branches near the primary inflorescence are considered major sinks and partial or complete removal of these lateral branches increased the number of flowers formed and pods set (Farrington & Pate, 1981; Porter, 1982). In the case of Bambara groundnut, many lateral branches are produced after the loss of apical dominance as early as 1 week after emergence and lateral branch elongation continued during development under long photoperiod. As lateral branch elongation and leaf growth continued during pod set, it can be speculated that assimilate demand by the pods was diminished, inhibiting pod enlargement and suppressing yield. Our results suggest that long photoperiod appears to be involved in controlling the balance of resource distribution via strategic modification of growth and the yield forming process. However, these suggestions deserve further analysis. Linnemann (1993) has shown through microscopic studies in Ankpa4 that embryo development under short (11.5 hr per day) and long (>14 hr per day) days was identical until about 17 days after flowering, but all embryos had slow growth. At approximately 41 days after flowering, while embryos in plants grown under short days increased rapidly to reach their maximum size, embryos in plants grown under long days ceased growth at about 17 days after flowering and eventually shriveled at about 32 days after flowering. This clearly supports the view that photoperiod gene action limits the partitioning of resources to the developing embryo. Several studies have revealed a genetic basis for abortion of developing seeds (Allphin, Wiens, & Harper, 2002; Cahrlesworth, 1989; Wiens et al., 1987). In soybean, fruit abortion postfertilization (no development) are associated with QTLs for flowering date, potentially placing the embryos at hazard with respect to environmental effects of heat stress and photoperiod (Tischner, Allphin, Chase, Orf, & Lark, 2003). The findings in soybean also showed that abortions at the later stage of partially developed embryos were associated with QTLs related to water stress or QTL that control the increase in number of ovules in a pod, placing a greater drain on the resources available to individual embryos. This might imply that limited translocation of photosynthate could be responsible for triggering abortion.

The number of pods and seed yield from long-day treatments considered in the ten Bambara groundnut genotypes was largely influenced by photoperiod as temperatures were kept consistent across all daylength conditions, although overall light energy received by the crop varied between treatments due to the increased vegetative growth of the crop under 16 hr photoperiod. The increase in vegetative biomass was only translated through into increased pod number and

yield in some of the genotype lines, with variation seen in harvest index between 12- and 16-hr, suggesting that this is not purely an effect of the amount of carbon available (Figure 4). Furthermore, the FutureCrop glasshouse conditions coupled with the fact that plants were grown as a crop stand makes the results from this study more comparable to natural conditions than previous studies. This is strongly supported by a previous field study in Botswana, which suggested that the onset of flowering in Bambara groundnut may be photoperiod insensitive, but pod set and pod filling can be completely aborted by long photoperiods (Harris & Azam-Ali, 1993). In sub-Saharan Africa, the crop is cultivated after the rains. In the event of later sowing dates arising from delays in rainfall, the risk of growing the crop during nonpermissive or marginal photoperiods could be eliminated by using IITA-686, Dip C, S19-3, and Uniswa Red because they are quantitative long-day types for pod and seed yield, with IITA also showing higher fertility under 16 hr. The level of variability for pod and seed yield observed in this study is a strong indication that the largest effects depended on both genetic and photoperiodic conditions. The genotypes identified in this study are being used to develop crosses for further dissection of the genetic control to help optimize photoperiod requirements to their production environments.

5 | CONCLUSION

Quantitative information on the influence of long photoperiod treatment on yield determining traits of Bambara groundnut is scarce. We have shown that there were strong photoperiodic effects on the yield component traits (pod number, pod weight, seed number, and seed weight—acting as proxies for pod set and pod filling) in Bambara groundnut and considerable differences were observed to exist between genotypes derived from different landraces under long photoperiods. In this study, the length of lateral branches and above-ground dry matter production was used as an indicator of vegetative sink strength and the number of pod and seed yield per plant as an indicator of reproductive sink capacity. Long photoperiod tended to abort or inhibit pod enlargement in the qualitative short-day genotype, reduce pod and seed number in quantitative short-day genotypes and increase pod and seed number in quantitative long-day genotypes, the latter being a new observation. The photoperiodic differences observed between fruit-set patterns of these three phenotypic classes might be explained by the sink strength (assimilate demand) of competing vegetative (lateral shoots tips, young leaves, and gynophores) and reproductive (flowers and fruits) organs. When elongated gynophores were produced in Ankpa 4 under the long photoperiod treatment, the abortion of embryonic pods and failure

in pod enlargement could also be an indicator of weak sink strength. These data show that pod development in Ankpa-4 may be controlled predominantly by the lateral shoots tips and gynophores' intense demand for assimilates (vegetative sink strength), which limits assimilate availability to the embryonic pods and hence pod abortion and/or growth inhibition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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