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Plant Growth Regulator-Brassinolide for Mitigating Field Waterlogging Stress on Maize

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Authors' contributions

This research was conducted in collaboration among all the authors. Author AI designed the study. Author VO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EE managed the analyses of the study. Author AP managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To assess the *pleiotropic* role of a plant growth regulator, commercially identified as brassinolide (BR) in mitigating waterlogging stress imposed on maize.

Study Design: A factorial combination of two maize varieties [Ikom White (IKW) and Oba-98], two BR levels (0 and 250 ml) and two waterlogging stages of maize growth [control (WL_0) and seedling stage (WL_1)], arranged as a split-split plot in a randomized complete block design with three replications was used.

Place and Duration of Study: Akpabuyo Local Government Area, Cross River State-Nigeria. A two-year field experiment was conducted during the dry seasons of December 2016 and December 2017.

Methodology: Waterlogging test was conducted on plots by demarcating them with 3.6 by 1.7 m metal sheets buried to a depth of 60 cm to prevent lateral soil-water movement. Two maize seeds were sown at 25 cm within and 75 cm between rows. The BR (250 ml) was sprayed foliar at 21 DAS. The non-waterlogging plots served as control. Observations were made on growth and yield variables as well as the plant's physiological traits.

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Results: Waterlogging significantly reduced the growth attributes of maize and increased ($p \leq 0.05$) the leaf moisture content. The photosynthetically active radiation on maize plants was substantially reduced ($p \leq 0.05$) by the waterlogging stress. Dry matter yield (DMY) and nutrient uptake in the leaves, stems and grains were reduced ($p \leq 0.05$) at both silking and at harvest. The effect of the BR was greater in Oba-98 with higher nutrient contents, radiation absorption, dry matter and grain yields than IKW.

Conclusion: Treatment of maize plants with BR could induce some tolerance of field waterlogging. Thus, for optimum efficiency in maize production under stressed soil condition of waterlogging, it is recommended that the foliar spray of BR at the 250 ml per plant rate be considered.

Keywords: Plant hormone; waterlogging stress; amelioration; hybrid maize; maize yield.

1. INTRODUCTION

Waterlogging constitutes serious environmental stress to crop production in soils developed on coastal plain sands in South-East Nigeria. Since the area is characterized by a high annual rainfall of about 3000 mm per annum, the soil is frequently too wet with insufficient oxygen for adequate respiration in roots [1], and the uptake of nutrients and water are adversely affected, causing a decline in crop production and increasing threat to food security [2]. Due to the close functional inter-dependence between roots and shoots, stress on roots from waterlogged soil threatens the shoot system by inhibiting nitrate uptake that arises from microbial denitrification and damage to uptake mechanisms from the absence of oxygen. Young leaves then obtain their nutrient from older leaves which then leads to premature senescence in the latter. Plants can wilt severely in bright light under waterlogged condition because of a lowered conductivity for water uptake that typifies oxygen deficiency in roots, thus producing a natural condition known as hypoxia (<21% oxygen) [3].

Brassinolide is a plant growth regulator derived from *Brassica,* a genus of plants in the mustard family (*Brassicaceae*). It is considered to strengthen the plant's immunity against various abiotic stresses by improving uptake and translocation of macro and micronutrients, thereby increasing plant growth and production [4]. The hormone regulates an array of physiological processes including cell expansion and proliferation, timing senescence, vascular differentiation and leaf development as well as giving some resistance to plants against various stressors [5]. In South-East Nigeria, production of maize has been hindered by periodic soil waterlogging and there is no scientific knowledge on exogenous plant hormone (particularly brassinolide) to improve maize growth and yield in waterlogged soil conditions. This is a new science in the study location.

The study was carried out to determine:

1. Brassinolide effect in ameliorating field waterlogging effect on the growth and yield of a hybrid and local maize varieties.
2. Effectiveness of brassinolide in supporting maize plant during the seedling growth stage under waterlogged soil condition.

2. MATERIALS AND METHODS

The experiment was conducted in two repeats for increased validity of data at Etak-Ukana in Akpabuyo Local Government Area (05º32´ and 04º57´ N; 07º15´ and 09º28´ E) of Cross River State-Nigeria, at the peak of dry season to avoid disturbance from natural precipitation during the waterlogging test. Akpabuyo soils are mainly acid sands [6] located in a humid tropical climate and rainforest vegetation characterized by high rainfall with distinct wet and dry seasons.

The composite soil samples (0-30 cm depth) from the site were analysed for routine physical and chemical properties. The pH was measured electrometrically using a pH meter in a 1:2.5 soil: water suspension. Total nitrogen was determined by the micro-Kjeldahl method [7]. The available phosphorus was extracted with acidic fluoride using the Bray P 1 method [8]; the phosphorus in the extract was determined colourimetrically by the molybdenum blue method of Murphy and Riley [9]. Organic carbon was determined by the dichromate wet oxidation method of Walkley and Black [10], the result was multiplied by VanBenmelen's factor of 1.724 [11] to estimate the soil organic matter. Exchangeable bases (calcium, potassium, magnesium and sodium) were extracted with 1N NH_4OA_C using 1:10 soil solution ratio. The potassium and sodium in the filtrate were determined by flame photometer, while calcium and magnesium were determined with an atomic absorption spectrophotometer (Model 6405 UV/ visible spectrophotometer, Jenway, UK).

The experiment comprised two maize varieties [Ikom White (V_I) and Oba-98 (V_2)], two waterlogging stages of maize growth [Control (WL_0) and the seedling stage (WL_1)], while the brassinolide was applied at two rates [without (0 ml) and with (250 ml)]. The main plots were assigned the two maize varieties; the waterlogging treatments were the subplots, while the sub-sub plots were the two brassinolide levels. The treatments were laid as a split-split plot in a randomized complete block design (RCBD) and replicated three times. The brassinolide was manufactured in the United Kingdom by Richblue Landbene Biochemical Company Limited, London. The Oba-98 maize variety is a hybrid type produced by Premier Seeds Company Limited, Kaduna-Nigeria, while Ikom White is a Nigerian indigenous maize variety grown locally under subsistence farming system.

2.1 Waterlogging Procedures

Waterlogging stress was simulated at 18 days after sowing (DAS) by demarcating each subplot for the test with 3.6 by 1.7 m metal sheets buried to a depth of 60 cm to effectively prevent lateral soil-water movement in different pools. The waterlogged plots were flooded continuously up to the marked (5 cm) level till full saturation was attained and maintained for 10-days.

2.2 Sowing

Two seeds were sown at a spacing of` 25 cm within and 75 cm between the rows and thinned to one plant per stand of 1 x 2.25 m at 2 weeks after sowing (WAS). The plots meant for waterlogging were flooded at 18 DAS from the water source to a 5 cm bank above the soil surface and maintained for 10-days based on the established flow rate.

Brassinolide is known to be polyhydroxy steroidal phytohormone that regulates several physiological processes in plants including seed germination, photo- morphogenesis, photosynthesis, flowering, sucrose translocation, assimilate partitioning, senescence and responses to different abiotic and biotic stresses [12]. It plays a vital role in protecting the translational machinery during waterlogging stress. The stress ameliorative properties of the hormone mitigate the harmful impacts of waterlogging on plants through the modulation of the components of the anti-oxidative defence system. It acts at very low concentration (10^{-9} M) and was diluted at the ratio of 1 ml to 1000 ml (BR and distilled water respectively), 250 ml was foliar-applied once per plant at 21 DAS, while the plots under normal irrigation were the non-waterlogging controls. At the end of the 10-day waterlogging treatment, the surface water was drained into a gutter through the UPVC waste pipes and the soil moisture recovered to normal in a few days, while the plants grew under normal irrigation till maturity. Nitrogen fertilizer as urea (46%) was applied basally in two splits, 14 and 38 DAS based on maize recommended rates [13]. Other field management practices including weeding and pest control were performed bi-weekly after sowing.

2.3 Observations

The growth parameters studied were plant height, the number of leaves, leaf area and leaf area index measured periodically (2, 4, 6 and 8 WAS). The specific leaf area was determined at silking. The sampling of above-ground biomass (stems, leaves and grains) was performed at heading (leaves) and harvest (stems, leaves and grains). The samples were oven-dried at 80°C for 48 hours and milled to estimate total nitrogen (N) content using the micro-Kjeldahl procedure (Jackson 1989). The photosynthetically active radiation ($\mu mol/m^2/s$) was determined by taking spot measurements periodically (4, 6, and 8 WAS) on the maize flag leaf during cloudless days, using the MQ-100: Quantum Integral Sensor with Handheld Meter (Apogee Instruments INC, USA). Relative water content in leaves (RWCL) was estimated at silking. The dry matter yield, harvest index and grain yield were determined at harvest. The relative growth rate (RGR) was measured at 2, 4 and 6 WAS, using the formula:

$$RGR = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1}$$

where:

ln =Natural logarithm
t_1=Time one (in days)
t_2=Time two (in days)
W_1=Total dry weight of plant at time one (g)
W_2=Total dry weight of plant at time two (g)

2.4 Statistics

The data were subjected to analysis of variance using the stat view software [14] to partition the effects of the three factors and their interactions. From the two repeats, the statistical comparison of data in the trials showed no significant difference, data from both sources were pooled for the analysis. Fisher's Protected Least Significant Difference (FPLSD) was used to compare treatment means at the 5% level of probability.

3. RESULTS

3.1 Soil Properties

The soil analysis showed that it had a pH (H_2O) of 5.4. The organic carbon content of the plough layer (0-30 cm) was 7.1 g/kg, organic matter was 12.2 g/kg, total nitrogen was 0.7 g/kg and Available Phosphorus was 47.67 mg/kg. The exchangeable base values (cmol/kg) were 1.93 for Calcium, 0.60 for Magnesium, 0.07 for Sodium and 0.12 for Potassium. There was a significant varietal, waterlogging and brassinolide effect on the soil chemical properties at harvest. Soils under Ikom White were significantly higher in nutrients than those under Oba-98. Conversely, soils planted with Oba-98 variety were higher ($P≤0.05$) in pH (5.9) than those of IKW (5.5). Irrespective of the maize variety planted, waterlogging stress significantly reduced the soil nutrient contents and increased the exchangeable acidity, whereas, the reverse occurred when BR was applied.

3.2 Plant Growth

The effects of varieties, waterlogging and brassinolide application on plant height, number of leaves, leaf area (LA) and leaf area index (LAI) are shown in Table 1. There was a significant varietal difference in plant height at 2 and 6 weeks after sowing (WAS) with IKW being taller than Oba-98, while Oba-98 was taller than IKW at 4 WAS. Waterlogging reduced ($P≤0.05$) plant height at all sampling periods, while BR enhanced ($P≤0.05$) it across the periods. The number of leaves was increased ($P≤0.05$) at 4, 6 and 8 WAS. Oba-98 produced more ($P≤0.05$) leaves at 4 WAS, while IKW had more ($P≤0.05$) leaves at 6 and 8 WAS. Waterlogging significantly reduced leaf number across the growth stages relative to the control (WL_0) while BR increased ($P≤0.05$) it at 4, 6 and 8 WAS. In successive sampling periods, there was no varietal effect on LA, but WL_1 significantly reduced the LA, while BR increased it. A similar trend occurred for LAI, however, BR increased the LAI at 4, 6 and 8 WAS (Table 1).

The interaction effects of waterlogging and brassinolide were significant for plant height, the number of leaves, LA and LAI. At 4 WAS BR better increased ($P≤0.05$) plant height under WL_0 than WL_1, while at 2 WAS, it significantly increased the number of leaves during WL_1 and reduced ($P≤0.05$) the same under WL_0. The BR enhanced ($P≤0.05$) the LA with or without waterlogging at 8 WAS, but it better increased ($P≤0.05$) the LAI under WL_l thanWL_0 at 6 WAS (Table 1).

3.3 Specific Leaf Area (SLA), the Relative Water Content in Leaves (RWCL), Relative Growth Rate (RGR) and Photosynthetically Active Radiation (PAR)

Table 2 shows the effects of varieties, waterlogging and brassinolide level on SLA, RWCL, RGR and PAR. Waterlogging reduced ($P≤0.05$) the SLA while BR enhanced it in both maize varieties. There was a significant increase in RWCL during waterlogging as compared with no waterlogging. The interaction effects of WS x BR were also significant, as BR reduced ($P≤0.05$) the RWCL during WL_l but increased ($P≤0.05$) it under WL_0 (Table 2). Across the growth stages, the RGR was significantly reduced in both varieties, but BR improved ($P≤0.05$) them considerably. For PAR, there was a considerable ($P≤0.05$) varietal, waterlogging and BR effects at 4, 6 and 8 WAS. The interaction effects of V x WS and WS x BR were also significant. Oba-98 absorbed more ($P≤0.05$) radiation (245.30 μmol/m^2/s) than IKW (167.22 μmol/m^2/s) at 4 WAS. Waterlogging significantly reduced the PAR at 4 and 6 WAS, while the application of BR increased ($P≤0.05$) it across the growth stages. The interaction effects of variety and waterlogging stages (V x WS) showed that Waterlogging more adversely ($P≤0.05$) affected PAR in IKW than in Oba-98 at 4 WAS, whereas, interactions of waterlogging and brassinolide (WS x BR) revealed that BR better improved ($P≤0.05$) PAR without waterlogging (Table 2).

3.4 Nitrogen (N) Contents

The effects for total N content in maize leaves, stems and grains are shown in Table 3. There

Table 1. Effect of waterlogging and brassinolide on growth, number of leaves, leaf area and leaf area index of two maize varieties at different sampling periods

Sources of variance	Plant height (cm)				Number of leaves				Leaf area (cm^2)				Leaf area index (LAI)			
Variety (V)	**2WAS**	**4WAS**	**6WAS**	**8WAS**	**2WAS**	**4WAS**	**6WAS**	**8WAS**	**2WAS**	**4WAS**	**6WAS**	**8WAS**	**2WAS**	**4WAS**	**6WAS**	**8WAS**
Ikom white (V_1)	6.06	23.70	54.64	117.25	5.42	6.58	9.25	12.75	31.71	145.47	425.49	569.44	0.02	0.08	0.19	0.29
Oba-98 (V_2)	4.67	26.78	51.22	115.63	5.08	7.83	8.50	10.92	37.04	170.48	354.48	534.46	0.02	0.09	0.23	0.26
LSD $_{(0.05)}$	1.08	1.27	2.43	NS	NS	0.84	1.01	1.06	NS	NS	NS	NS	NS	NS	NS	NS
Waterlogging stages (WS)																
WL_0	6.53	31.85	65.27	130.20	5.50	8.25	10.92	14.25	43.81	199.51	498.53	659.41	0.02	0.10	0.28	0.34
WL_1	4.19	18.63	40.59	102.68	5.00	6.17	6.83	9.42	24.94	116.44	281.45	444.49	0.01	0.07	0.14	0.20
LSD $_{(0.05)}$	1.08	1.27	2.43	2.36	0.41	0.84	1.01	1.06	9.78	43.01	106.48	78.43	0.01	0.02	0.05	0.04
Brassinolide (BR)																
G_0	4.71	22.63	46.61	110.93	5.17	5.92	7.33	10.83	28.88	104.92	301.58	429.35	0.02	0.06	0.18	0.24
G_1	6.01	27.85	59.25	121.95	5.33	8.50	10.42	12.83	39.87	211.03	478.40	674.54	0.02	0.11	0.24	0.31
LSD $_{(0.05)}$	1.08	1.27	2.43	2.36	NS	0.84	1.01	1.06	9.78	43.01	106.48	78.43	NS	0.02	0.05	0.04
WS x BR																
WL_0 G_0	6.15	30.00	57.85	124.30	5.83	9.33	9.67	14.00	39.84	150.14	443.17	605.83	0.02	0.08	0.28	0.03
WL_0 G_1	6.91	33.70	72.70	136.10	5.17	9.17	12.17	14.50	47.79	248.87	553.88	712.98	0.02	0.13	0.28	0.36
WL_1 G_0	3.27	15.25	35.37	97.55	4.50	4.50	5.00	7.67	17.93	59.69	159.98	252.87	0.01	0.04	0.08	0.15
WL_1 G_1	5.11	22.00	45.80	107.80	5.50	7.83	8.67	11.17	31.96	173.18	402.92	636.10	0.02	0.09	0.19	0.25
LSD $_{(0.05)}$	NS	1.79	NS	NS	0.58	NS	NS	1.50	NS	NS	NS	110.91	NS	NS	0.07	NS

NS = not significant; WL_0 = non-waterlogging control; WL_1 = waterlogging at seedling stage; G_0 = without brassinolide (0 mL); G_1 = with brassinolide (250 mL)

Table 2. Effect of waterlogging and brassinolide on specific leaf area (SLA), the relative water content in leaves (RWCL), relative growth rate (RGR) and photosynthetically active radiation (PAR) of two maize varieties

Treatment	SLA at silking (cm^2/g)	RWCL at silking	RGR(g g^{-1}/day)			PAR (µmol/m^2/s)		
Variety (V)			**2WAS**	**4WAS**	**6WAS**	**4WAS**	**6WAS**	**8WAS**
Ikom white (V_1)	249.98	0.42	0.08	0.04	0.02	167.22	196.58	276.19
Oba-98 (V_2)	275.51	0.47	0.08	0.05	0.02	245.30	290.11	367.64
LSD $_{(0.05)}$	NS	NS	NS	NS	NS	52.27	NS	NS
Waterlogging stages (WS)								
WL_0	324.05	0.33	0.09	0.06	0.02	314.42	322.14	388.89
WL_1	201.44	0.56	0.07	0.04	0.01	98.11	165.25	254.94
LSD $_{(0.05)}$	54.62	0.12	0.01	0.01	0.01	52.27	107.28	NS
Brassinolide (BR)								
G_0	196.16	0.49	0.07	0.04	0.01	145.81	165.14	205.11
G_1	329.33	0.40	0.09	0.06	0.02	266.72	322.25	438.72
LSD $_{(0.05)}$	54.62	NS	0.01	0.01	0.01	52.27	107.28	148.74
V x WS								
V_1 WL_0	308.39	0.30	0.09	0.05	0.02	241.44	238.78	322.44
V_1 WL_1	191.57	0.54	0.07	0.03	0.01	93.00	154.39	229.94
V_2 WL_0	339.71	0.36	0.09	0.06	0.02	387.39	405.50	455.33
V_2 WL_1	211.31	0.58	0.07	0.04	0.01	103.22	176.11	279.94
LSD $_{(0.05)}$	NS	NS	NS	NS	NS	70.08	NS	NS
WS x BR								
WL_0 G_0	256.44	0.29	0.08	0.05	0.02	200.89	214.22	238.55
WL_0 G_1	391.66	0.38	0.10	0.06	0.03	427.94	430.05	539.22
WL_1 G_0	135.89	0.70	0.06	0.03	0.01	90.72	116.06	171.67
WL_1 G_1	266.99	0.43	0.08	0.05	0.02	105.50	214.44	338.22
LSD $_{(0.05)}$	NS	0.17	NS	NS	NS	70.08	NS	NS

NS = not significant; WL_0 = non-waterlogging control; WL_1 = waterlogging at seedling stage; G_0 = without brassinolide (0 mL); G_1 = with brassinolide (250 mL)

was a significant varietal difference in N content across the plant materials as Oba-98 contained more N than IKW in all the growth stages. Waterlogging reduced (*P≤0.05*) the N content in all plant parts but BR application increased (*P≤0.05*) it at both silking and at harvest (Table 3). The interactions among V x BR and WS x BR showed that BR increased (*P≤0.05*) the N content in leaves at silking especially under no waterlogging. At harvest, the BR increased (*P≤0.05*) the N contents in the leaves of both maize varieties, with or without waterlogging. The interaction of WS x BR showed significance in grains at harvest as the BR increased (*P≤0.05*) the N content in grains under the waterlogging and non-waterlogging soil conditions.

3.5 Leaf Dry Weight (LDW), Stem Dry Weight (SDW), Harvest Index (HI) and Grain Yield (GY)

Waterlogging reduced (*P≤0.05*) the LDW of both maize varieties, whereas, the BR significantly increased them (Fig. 1). The SDW of maize was also significantly reduced by the waterlogging treatment, however, the beneficial role of brassinolide boosted (*P≤0.05*) the SDW of both varieties (Fig. 2). The interaction effects of WS x BR for SDW showed that BR-induced increase (*P≤0.05*) in SDW was more pronounced without waterlogging (Fig. 2).

No major differences between varieties were found for harvest index (HI). However, the waterlogging conditions significantly reduced the HI, while BR increased (p≤0.05) it (Fig. 3). Waterlogging plus BR had a significant effect on the cob filling and cob size of both maize varieties as well as their grain yield. Oba-98 significantly (p≤0.05) out-yielded (1.70 t/ha) IKW (1.53 t/ha) as shown in Fig. 4. However, waterlogging reduced (p≤0.05) grain yield, but BR significantly increased the yield despite waterlogging stress, especially for Oba-98. The interaction effects of V x WS and WS x BR showed that Oba-98 was a better ($p \leq 0.05$) grainer (1.24 t/ha) than IKW (1.15 t/ha) during waterlogging at the seedling stage (WL_1) of maize, but the use of BR improved ($p \leq 0.05$) the yield of both varieties during the waterlogging stress (Fig. 5).

Table 3. Nitrogen (N) contents (g/kg) in leaves at silking, stems and grains at harvest

Treatment	Leaf-N at silking	Leaf-N at harvest	Stem-N at harvest	Grain-N at harvest
Variety (V)				
Ikom white (V_1)	30.90	16.48	18.56	23.78
Oba-98 (V_2)	32.11	19.86	20.20	25.88
LSD $_{(0.05)}$	0.54	0.25	0.95	0.73
Waterlogging stages (WS)				
WL_0	32.68	20.59	22.64	30.84
WL_1	30.32	15.75	16.12	18.81
LSD $_{(0.05)}$	0.54	0.25	0.95	0.73
Brassinolide (BR)				
G_0	29.03	15.30	16.16	17.95
G_1	33.97	21.04	22.60	31.70
LSD $_{(0.05)}$	0.54	0.25	0.95	0.73
V x WS				
V_1 WL_0	31.93	18.26	21.70	29.45
V_1 WL_1	29.87	14.69	15.41	18.10
V_2 WL_0	33.43	22.91	23.59	32.24
V_2 WL_1	30.78	16.81	16.82	19.52
LSD $_{(0.05)}$	NS	0.36	NS	NS
V x BR				
V_1 G_0	28.00	13.28	15.42	17.11
V_1 G_1	33.80	19.68	21.69	30.44
V_2 G_0	30.07	17.32	16.89	18.79
V_2 G_1	34.15	22.39	23.52	32.96
LSD $_{(0.05)}$	0.76	0.36	NS	NS
WS x BR				
WL_0 G_0	30.65	18.02	19.38	22.44
WL_0 G_1	34.72	23.15	25.91	39.25
WL_1 G_0	27.42	12.52	12.93	13.47
WL_1 G_1	33.23	18.92	19.30	24.15
LSD $_{(0.05)}$	0.76	0.36	NS	1.03
V x WS x BR				
V_1 WL_0 G_0	29.27	14.01	18.18	21.04
V_1 WL_0 G_1	34.60	22.52	25.22	37.86
V_1 WL_1 G_0	26.73	12.55	12.67	13.19
V_1 WL_1 G_1	33.00	16.84	18.50	23.01
V_2 WL_0 G_0	32.03	22.03	20.58	23.85
V_2 WL_0 G_1	34.83	23.78	26.59	40.63
V_2 WL_1 G_0	28.10	12.61	13.19	13.74
V_2 WL_1 G_1	33.47	21.00	20.45	25.30
LSD $_{(0.05)}$	NS	0.50	NS	NS

NS = not significant; WL_0 = non-waterlogging control; WL_1 = waterlogging at seedling stage; G_0 = without brassinolide (0 mL); G_1 = with brassinolide (250 mL)

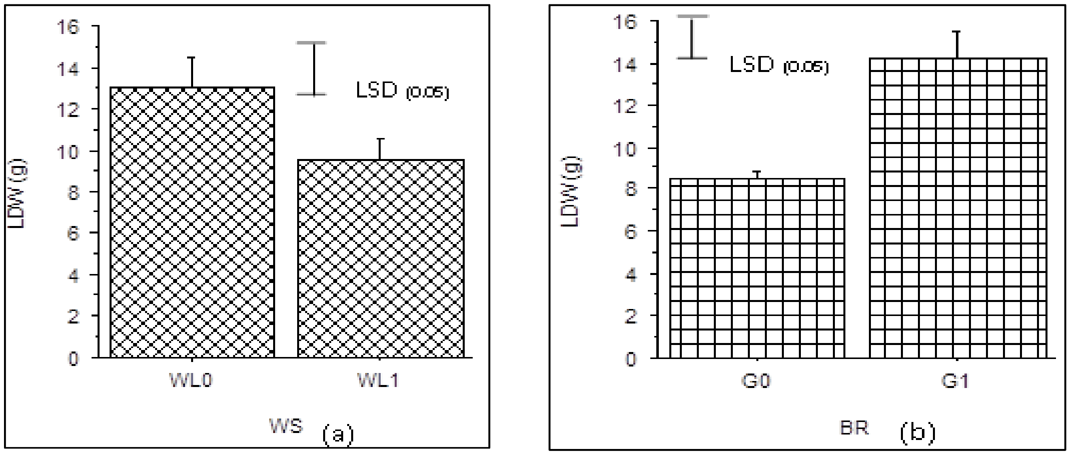


Fig. 1. Waterlogging (a) and brassinolide (b) effects on leaf dry weight of maize

LDW= leaf dry weight; WS= waterlogging stages; WL0= non-waterlogging control; WL1= waterlogging at seedling growth stage of maize; BR= brassinolide application; G0= without brassinolide (0 ml); G1= with brassinolide (250 ml)

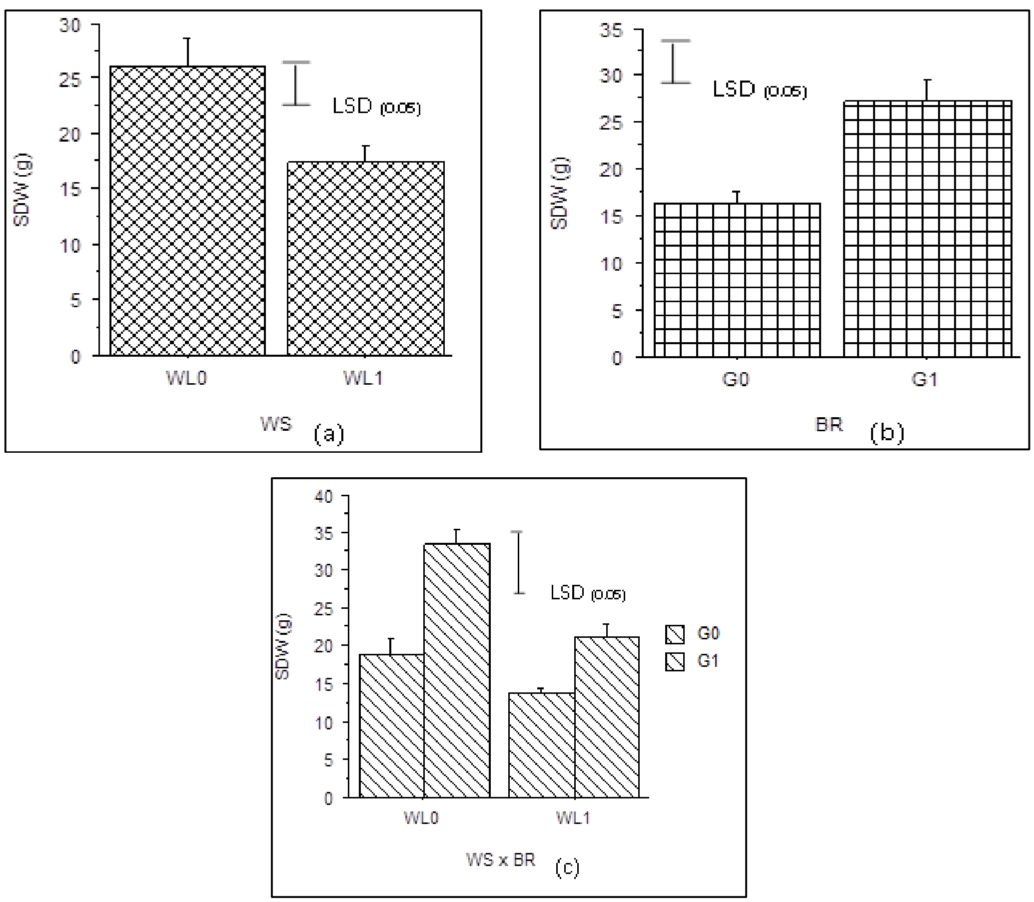


Fig. 2. Main and interaction effect of waterlogging (a), brassinolide (b) and WS x BR on stem dry weight of maize

SDW= Stem dry weight; WS= waterlogging stages; WL0= non-waterlogging control; WL1= waterlogging at seedling growth stage of maize; BR= brassinolide application; G0= without brassinolide (0 ml); G1= with brassinolide (250 ml)

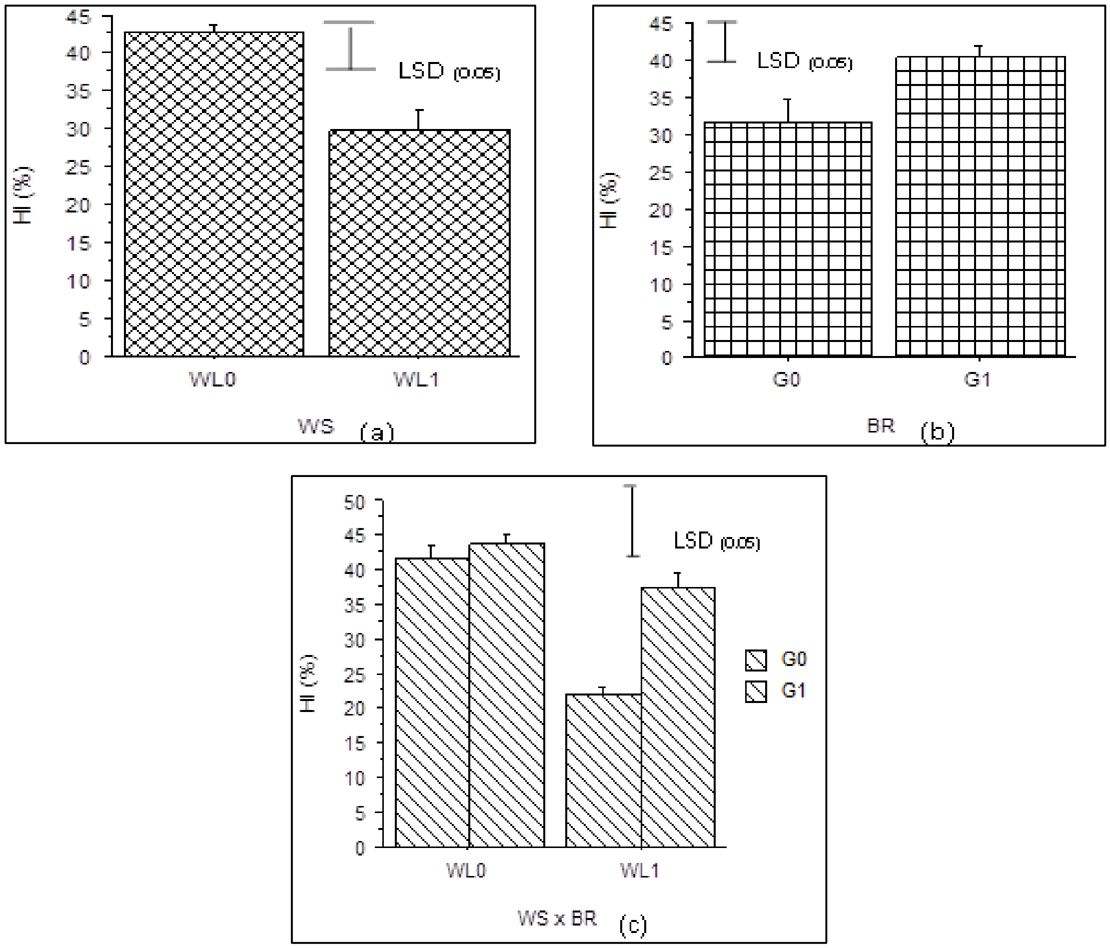
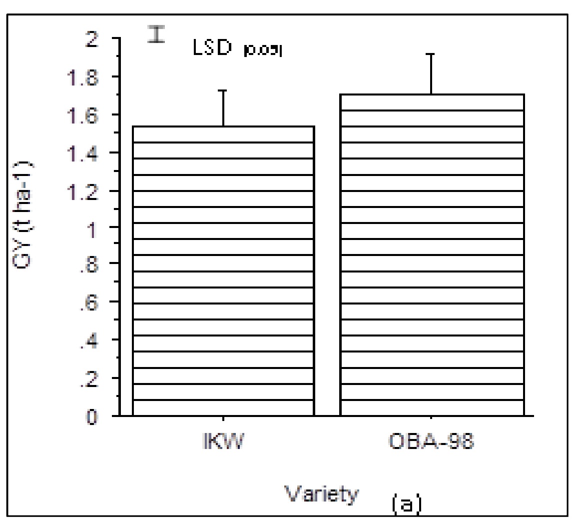


Fig. 3. Main and interaction effect of waterlogging (a), brassinolide (b) and WS x BR (c) on harvest index of maize

HI= Harvest index; WS= waterlogging stages; WL0= non-waterlogging control; WL1= waterlogging at seedling growth stage of maize; BR= brassinolide application; G0= without brassinolide (0 ml); G1= with brassinolide (250 ml)



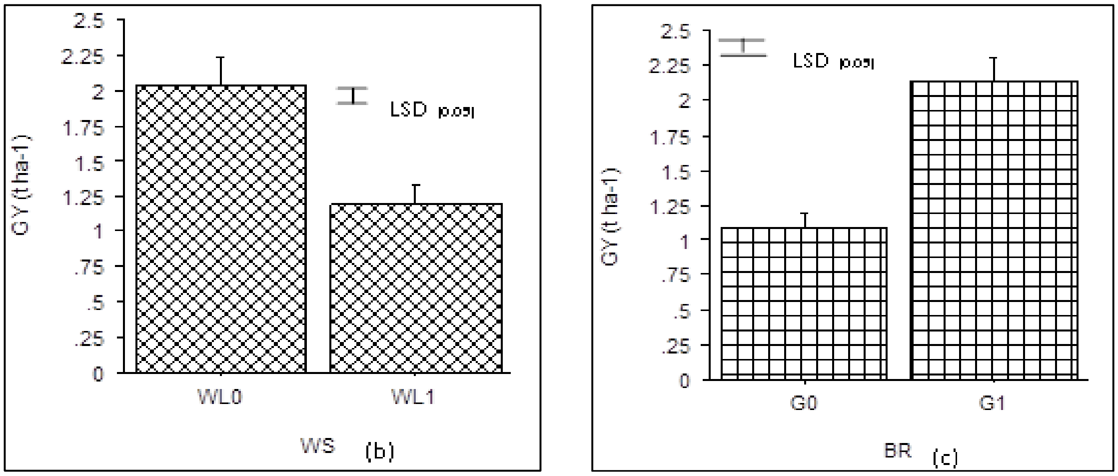


Fig. 4. Varietal (a), waterlogging (b) and brassinolide (c) effects on grain yield of maize
GY= Grain yield; WS= waterlogging stages; WL0= non-waterlogging control; WL1= waterlogging at seedling growth stage of maize; BR= brassinolide application; G0= without brassinolide (0 ml); G1= with brassinolide (250 ml)

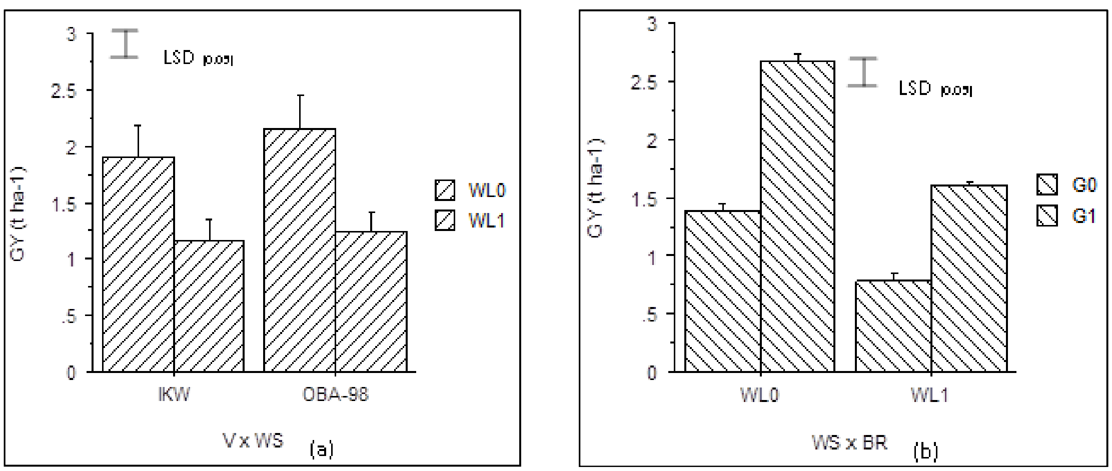


Fig. 5. Interaction effects of V x WS (a) and WS x BR (b) on grain yield of maize
GY= Grain yield; WS= waterlogging stages; WL0= non-waterlogging control; WL1= waterlogging at seedling growth stage of maize; BR= brassinolide application; G0= without brassinolide (0 mL); G1= with brassinolide (250 mL)

4. DISCUSSION

4.1 Soil Properties

Waterlogging stress significantly reduced the nutrient contents [(organic matter, total nitrogen, available phosphorus and exchangeable bases (Calcium, Magnesium, Potassium, Sodium)] of the soil possibly because of the reduced condition of the soil environment due to the depletion of oxygen by plant roots and soil microbes as well as the consequent competition for oxygen by both, leading to hypoxic or even anoxic soil environment [15]. Application of brassinolide facilitated the release and utilization of these nutrients irrespective of the moisture stress possibly due to its source to sink tendencies and its ability in efficient responses to soil abiotic stressors [16]. The pH in Oba-98 plots was significantly higher than those of IKW which could be attributed to increased microbial activities that facilitated the transformation of N for higher nutrient availability and uptake. Soil enzyme activity and microbial community diversity have often been proposed as important indicators following the restoration of soil function

[17]. Conversely, soils under IKW were higher in nutrients than those of Oba-98 possibly due to higher nutrient uptake in the latter relative to the former based on BR ability in exploring the genetic and physiological potentials of the hybrid maize in cell division and elongation for utilization of soil nutrients more efficiently [18].

4.2 Plant Growth

The morphological growth characteristics (plant height, number of leaves, leaf area and leaf area index) were adversely affected by waterlogging. Ikom white tolerated the stress better than Oba-98. This could be due to the increased availability of soluble sugar, that increased the activity of glycolytic pathway and fermentation enzymes which enhanced the antioxidant defence mechanism in the variety to boost its immunity against the oxidating stress induced by waterlogging [19]. Waterlogging suppressed plant growth substantially possibly because of the reduction of oxygen below the optimal level for plant metabolic processes [20]. However, the brassinolide (BR) was able to improve growth possibly through its nucleic acid and soluble carbohydrate contents that regulate cell division and elongation for growth and defence [21] in plants.

The specific leaf area (SLA) of both maize varieties was greatly subdued during waterlogging possibly because of the anaerobic environment that produced low adenosine triphosphate (ATP) per glucose molecules [22]. The increase in SLA through the application of BR clearly showed that the BR possibly induced the chlorophyll content in the maize foliage as reported in [23].

4.3 Relative Water Content in Leaves (RWCL), Relative Growth Rate (RGR and Photosynthetically Active Radiation (PAR)

The relative water content in the leaves (RWCL) was high during waterlogging. This observation is contrary to a previous report [24] where waterlogging significantly reduced the relative water content and membrane stability in leaves. The BR reduced the RWCL during waterlogging which is consistent with the report of Rao et al. [25] that BR can increase resistance in plants against waterlogging stress. There was a decline in the relative growth rate (RGR) during waterlogging. This could be due to the lower nitrogen concentration induced by the stress in the maize stems [26]. However, the growth was ultimately enhanced by BR application possibly because of its growth enhancement effect [27].

The hybrid maize (Oba-98) was capable of absorbing higher radiation (245.30 $\mu mol/m^2/s$) than IKW (167 $\mu mol/m^2/s$) suggesting that its superiority in photosynthetic performance influenced its ability in maintaining higher energy status via fermentation during the waterlogging stress [28]. Consequently, the decreased PAR under waterlogging could be attributed to stomatal closure [29], decrease in leaf chlorophyll concentration [30] and ethylene production [31]. The BR boosted radiation absorption under the stressed soil condition probably because of its ability in regulating photosynthesis in plants [16]. It seems that Oba-98 could respire and exchange gases such as carbon dioxide and oxygen for photosynthesis than IKW despite the flooded condition of the soil. This could be due to its anatomical and morphological adaptative features [32] as a hybrid type. More so, the interaction effects between waterlogging stage and brassinolide were indicative that the BR increased the radiation absorbing capability of both maize varieties possibly because of its functions in regulating physiological processes of enzymatic acceleration as well as photosynthetic activities in plants as observed in Yu, et al. [23]. At 6 WAS, waterlogging significantly reduced PAR in both maize varieties suggesting that there was a reduction in total chlorophyll in their leaves [33]. The BR enhanced PAR absorption which may have contributed to mitigating the negative effect of waterlogging on plant vegetative and reproductive growth [34].

4.4 Nitrogen (N) Contents

Nitrogen is an essential plant nutrient for growth was partitioned to the leaves at silking than at harvest due mainly to its availability and greater supply to plants at that stage for dry matter accumulation in the developing sink [35]. Waterlogging adversely reduced the N content across the plant materials indicating that there was a reduction in oxygen concentration that retarded uptake in the root zone, thereby impairing plant metabolism under the anaerobic soil condition [36]. The plant partitions and grains treated with BR had the highest N-content, suggesting that the hormone not only increased metabolic activities and grain yield in BR-treated maize but also enhanced better nutrient uptake for the revival of the plants [4] under the flooded condition.

4.5 Leaf Dry Weight (LDW), Stem Dry Weight (SDW), Harvest Index (HI) and Grain Yield (GY)

The leaf and stem dry weight was substantially reduced under waterlogging relative to the control, possibly due to impairment of water-absorbing ability of the plants through the reduction in leaf turgidity as well as translocation of dry matter [37]. However, it is possible that the BR increased the dry matter content of both varieties through the promotion of active cell division, elongation and enlargement as reported in Muthukumar, et al. [38] for enhanced vegetative growth. Waterlogging significantly reduced the HI of both maize varieties, implying substantial reductions in biological and economic yields [39]. The BR increased the HI because of its ability to increase the mobilization and translocation of metabolites [40] to the sink which contributed to higher grain yield.

The reduction in grain yield under waterlogging could be due to oxygen deficiency and the anaerobic conditions that inhibited rhizospheric activities [41]. Oba-98 had a higher yield (1.70 t/ha) than IKW (1.53 t/ha) which could be due to their more robust root system [42], while the BR increased the yield possibly because of its ability for improved productivity [5].

5. CONCLUSION

This study clearly showed that the growth attributes of both maize varieties were adversely affected by waterlogging. Ikom White was superior to Oba-98 in growth attributes but Oba-98 was more efficient in N-uptake, better yielding and produced grains than the local IKW variety. Reductions in photosynthetically active radiation and grain yield were significantly ameliorated by the BR treatment under the stressed soil condition. The N-uptake, RGR, dry matter yield, harvest index and moisture content were adversely affected by the stressor. Both biological and economic yields of the maize varieties were enhanced as the BR induced some tolerance through its ability to promote metabolic activities as well as nutrient and water uptake by the plants during the waterlogging stress. Thus, the beneficial role of the BR will be more optimized under stressed soil condition of waterlogging in the study area and other parts of the world with the same challenge for improved and sustained soil fertility and food security.

Thus, for optimum efficiency in maize production under stressed soil condition of waterlogging, it is recommended that the foliar spray of BR at the 250 ml per plant rate be considered for improved and sustained soil fertility, increased yield of hybrid and local maize varieties and to meet the increasing demand by the growing population.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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