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Effects of plant growth regulators and NaCl on early developmental stages of *Striga hermonthica*

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Abstract

A series of laboratory experiments were carried out in order to investigate the effect of plant growth regulators (PGRs) and NaCl on germination and haustorium initiation of *Striga hermonthica*. Therefore, manipulation of seed germination and pre-attachment stages are mandatory for successful management. In the present study, conditioned *Striga* seeds in water treated with 2, 4-D produced high germination 76% in response to GR24. Induction of germination by BAP varied from low (30%) to moderate (50%). Moreover, conditioned seeds treated with the combination of auxins and NaCl reduced germination by 90-100% as compared to the control. Furthermore, germination of *Striga* was relatively higher in the presence of kinetine and BAP in presence or absence of the GR24. In addition, both the cytokinies in combine with NaCl induced haustorium initiation in a dose dependent manner. Both axuins in combination with NaCl displayed negative response for haustorium initiation, in absence of haustorium factor. Striga seeds conditioned in presence of PGRs plus NaCl and subsequently treated with germination stimulant displayed considerable germination reduction (35-100%). Kinetine in combination with NaCl completely inhibited *Striga* germination in response to the synthetic germination stimulants GR24 in a concentration dependent manner. Withrespect to haustorium, all treatments reduced *Striga* germilings, significantly as compared to control (88%) in response to DMBQ. Auxin, 2, 4-D applied to Striga seeds after seeds conditioned in water was completely inhibited (100%) haustoria as compared to the control. Furthermore, Osmotic potential may significantly affect germination and haustorium initiation of *Striga hermonthica*. The results are consistent with a model in which both germination and subsequent morphogenesis in *Striga* are associated with exogenous and endogenous phytohormones.

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Introduction

The plant hormones, auxins and cytokinins, are involved in several stages of plant growth and development such as cell elongation, cell division, tissue differentiation, and apical dominance. The biosynthesis and the underlying mechanism of auxins and cytokinins action are subjects of intense investigation (Frankenberger and Arshad, 1995; Casanova *et al.*, 2004). Cytokinins as plant hormones acting in conjunction or in opposition to other plant hormones, play a major role throughout the development from seed germination to leaf and plant senescence. They modulate physiological processes important throughout the life of the plant including photosynthesis and respiration. They are believed to be actively involved in breaking seed dormancy. They also inhibit oxidation, preventing the sharp rise in respiration occurring during senescence.

Root parasitic weeds generally damage their hosts plant even before they emerge above ground. Yield losses in West African cereals due to *Striga* species have been estimated to average 24%, but total loss can occur in some years in areas of heavy infestation (Babiker et al., 2000). The life cycle of *Striga hermonthica* is closely linked to its host. In order to germinate, a Striga seed has to be in a warm moist environment for several days (conditioning) prior to exposure to an exogenous stimulant (Hassan *et al.*, 2009). Induction of germination in the absence of, or away from a host, suicidal germination lead to depletion of seed reserves in the soil. Several germination stimulants were identified from host and non host plants, and many of their analogues have been synthesized and proved to be effective (Matusova *et al.*, 2005). Hassan *et al.*, (2010) reported that *Striga* germination was significantly decreased with increasing NaCl solution, irrespective to their concentration or conditioning status.

Cytokinins have been found to be the most limiting in *Striga*-infected host and it was hypothesized that exogenous application could disrupt the imbalance and possibly reverse the negative effect of *Striga* on sorghum photosynthesis (Wirnkar, 1998). In that event exogenous cytokinins compensated for the endogenous cytokinins lost to the parasite. *Striga* depends upon the host for the cytokinins required for normal plumule development (Doggett, 1988). Moreover, Cytokinin stimulated activity of l-aminocyclopropane-1-carboxylic acid (ACC) oxidase in *Striga asiatica* seeds (Babiker *et al.*, 1993).

Plant hormones are probably also necessary for the haustoria formation, which is the next specific developmental stage of parasitic plants from the genus *Striga*(Keyes *et al.*, 2001). Plant hormones are extremely important for the regulation of seed dormancy,germination and developmental stages (Finkelstein, 2004; Kucer *et al.*, 2005). Hassan *et al.*, (2011) reported that morphogenesis of cultured *S. hermonthica* is influenced by exogenous growth regulators. The objective of this study was to investigate the effects of NaCl, auxins and cytokinins on *Striga* germination and haustorim initiation in presence or absence of chemicals stimulants.

Materials and methods

A series of laboratory experiments were undertaken, in a completely randomized design with four replicates, to determine the effects of Plant Growth Regulators (auxin, cytokinine) and NaCl on *Striga* germination and haustorium initiation during or after seeds conditioning in response to germination stimulant. Experiments were repeated at least once to confirm the previous results.

Striga hermonthica seeds, used in this study, were collected from parasitic plants growing under sorghum in 2004 at the Gezira Research Station Farm in Wad Madani. *S. hermonthica* seeds were surface disinfected as previously described by Hassan et al., (2009).

Chemicals used were auxins (naphthalene acetic acid (NAA) and 2,4-dichloro-phenoxyacetic acid (2,4-D), cytokinines (Benzylamino purine (BAP), and Kinetin) obtained from Tissue Culture Department, Commission of Biotechnology and Genetic

Engineering (CBGE), while NaCl was purchased from the local market.

GR24 was provided by Professor B. Zwanenberg, the University of Nimijhen, the Netherlands. A stock solution of the stimulant was prepared by dissolving 1 mg in 1 ml of acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept in a fridge at 5 ºC till used. DMBQ was a gift from Professor Sugimoto, Y. from Kobe University, Japan.

Effects of PGRs and NaCl applied during conditioning on seeds germination

Striga seeds were conditioned as described by Hassan et al., (2009). Sterilized glass fiber filter papers (GF/C) discs (8 mm diameter) were placed in 9 cm Petri dishes lined with glass fiber filter papers. Discs were moistened with 5 ml distilled water, or growth regulators (auxins and cytokinines) at 2, 4, 6 and 8% or NaCl at 3.3 and 6.3 µM. About 25-50 surface disinfected *S. hermothica* seeds were sprinkled on each of the glass fiber discs in each Petri dish. The dishes, sealed with parafilm, placed in black polythene bags were incubated at 30 ºC in the dark for 10 days.

For germination bioassay glass filter paper discs containing *Striga* seeds were dapped on filter paper (Whatman No. 1) to remove excess water and then transferred to sterile Petri dishes. Each disc was treated with 20 µl, of the respective test solution (GR24) at 0.1 ppm the seeds were reincubated in the dark at 30 ºC and then examined for germination 24 h later using a stereomicroscope.

Effects of PGRs and NaCl applied during conditioning on S. hermonthica haustorial initiation

The experiment was conducted at Environmental and Natural Resources Research institute (ENRRI) laboratory. In this experiment, a number of plant growth regulators (PGRs) and NaCl were screened for their effects on haustrorium initiation. Surface sterilized *Striga* seeds, placed on 8 mm glass fiber discs conditioned in presence and absence of PGRs as described above were blotted dry on filter papers (Whatman No. 1) and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with 20 µl GR24 solution (0.1 ppm) to induce germination. The Petri dishes sealed with parafilm and placed in black polyethylene bags, were incubated in the dark at 30 ºC for 48h. The discs containing the germinated seeds (*Striga* germilings) damped on a filter paper, were placed, and inverted top-down on similar discs without *Striga* seeds. The Pairs of discs were treated either with 40 µl solution of 2, 6-dimethoxybenzoquinone (DMBQ) (10 µM). *Striga* germilings resulting from seeds conditioned in distilled water similarly treated with DMBQ were included as controls for comparison. The Petri dishes, sealed with Parafilm and placed in polyethylene bags, were incubated in the dark at 30 ºC for an additional 24 h and then examined for haustorium initiation using a binocular stereomicroscope.

Effects of PGRs in combination with NaCl on Striga germination

Discs containing pre-conditioned *Striga* seeds, blotted dry on filter paper to remove excess water, were placed in 9 cm Petri dishes lined with glass fiber filter papers (GFFP). Conditioned *Striga* seeds, on discs treated with 20 µl with PGRs or NaCl in combination with synthetic germination stimulant GR24 in a ratio 1:1. A conditioned *Striga* seeds in water and treated with GR24 (20 µl/disc) was included as control for comparison. The Petri dishes were sealed with Parafilm, wrapped in aluminum foil, and incubated at 30ºC ± 2 in the dark for 24 h. Subsequently, seeds were examined for germination under a stereomicroscope. A seed was considered germinated when the radical protruded from the seed coat.

Effects of PGRs in combination with NaCl on haustorial initiation

Striga seeds on glass fiber filter papers were conditioned and induced to germinate with GR24 as previously described. Discs containing germinated *Striga* seeds, (Striga germiling), were blotted dry on filter paper and placed top-down on GFFP without seeds. Each pair of discs was treated with 40 µl of PGRs or NaCl in absence DMBQ. Discs containing

Striga germiling were conditioned in distill water treated with 40 µl DMBQ (20 µM) were included as control for comparison. The Petri dishes were sealed with Parafilm, wrapped in aluminum foil and incubated at 30 ±2 ºC in the dark for prior to assessment of treatment effects on haustorium induction under a stereomicroscope.

Data on percentage germination and haustorial initiation were calculated for each discs transformed to arcsine (Gomez and Gomez. 1984) and subjected to analysis of variance (ANOVA). Means were tested for significance with the Duncan Multiple Range Tests at 5%.

Results

Effects of PGRs, on S. hermonthica seeds germination

Striga seeds treated with distilled water displayed negligible germination (data not shown). Germination percentage of conditioned seeds treated with GR24 at 0.1ppm was 73% (Fig 1a). Auxines displayed moderate to high activity (46 - 76% germination), irrespective to their concentrations. 2, 4-D displayed high germination (76%) at the lowest concentration of it. A further increase in concentration of 2, 4-D reduced germination considerably. NAA at the lowest concentration (2%) reduced 65% germination as compared to the control. Increasing concentration to 8% of the NAA elicited germination comparable to the lowest concentration in response to GR24. However, the cytokinine kinetine at 2% elicited germination of *Striga* seeds germination (66%) in absence to GR24. While at the highest concentration, it reduced germination by 34% as compared to the control. Results displayed that the two hormones 2, 4-D and NAA in absence of GR24, reduced germination by 90-100% (Fig 1a).

Furthermore, plant growth regulators affected on *Striga* radical length. Cytokinine kinetine displayed the longest (5.9 - 6.3cm.) radical in presence or absence of GR24 as compared to other treatments and the control (5.9 cm.) (Fig 1 c).

Moreover, results displayed that cytokines kinetine and BAP in presence or absence of GR24, induced haustorium initiation in absence of DMBQ (Fig 1b). A further increase in concentration of both cytokines reduced haustorium considerably. However, auxins NAA and 2, 4-D treated to *Striga* seed displayed no haustorium initiation.

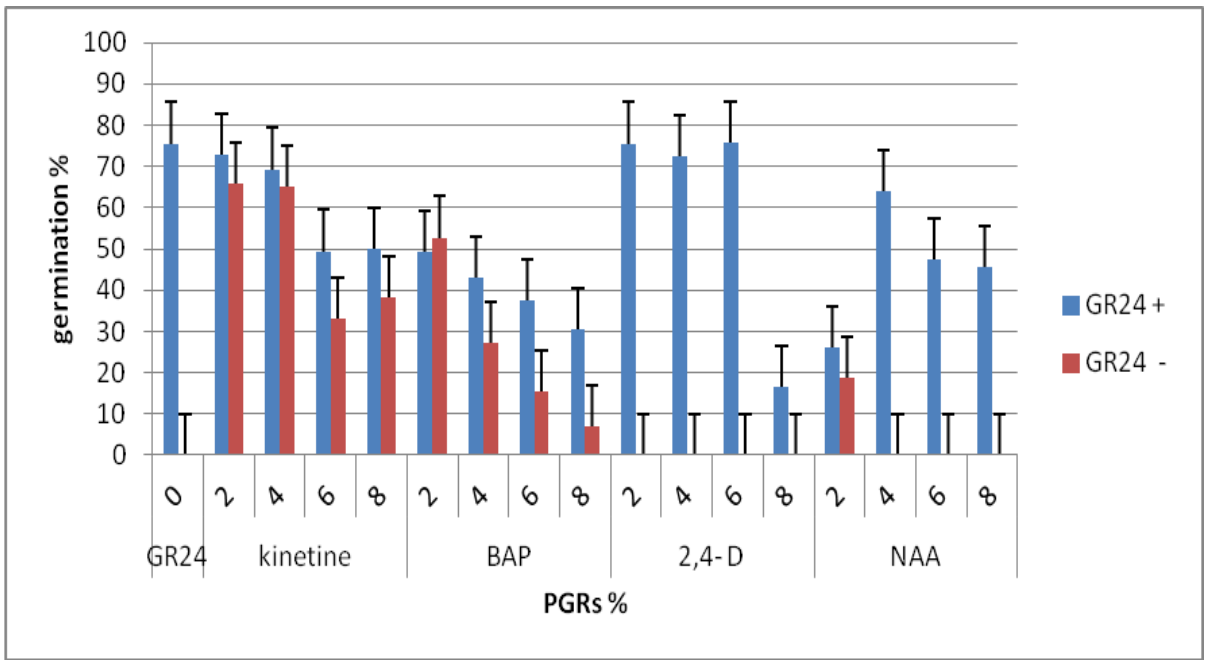

Fig. 1a. Germination percentage of S. hermonthica seeds pre-conditioned in water, exposed to PGRs.

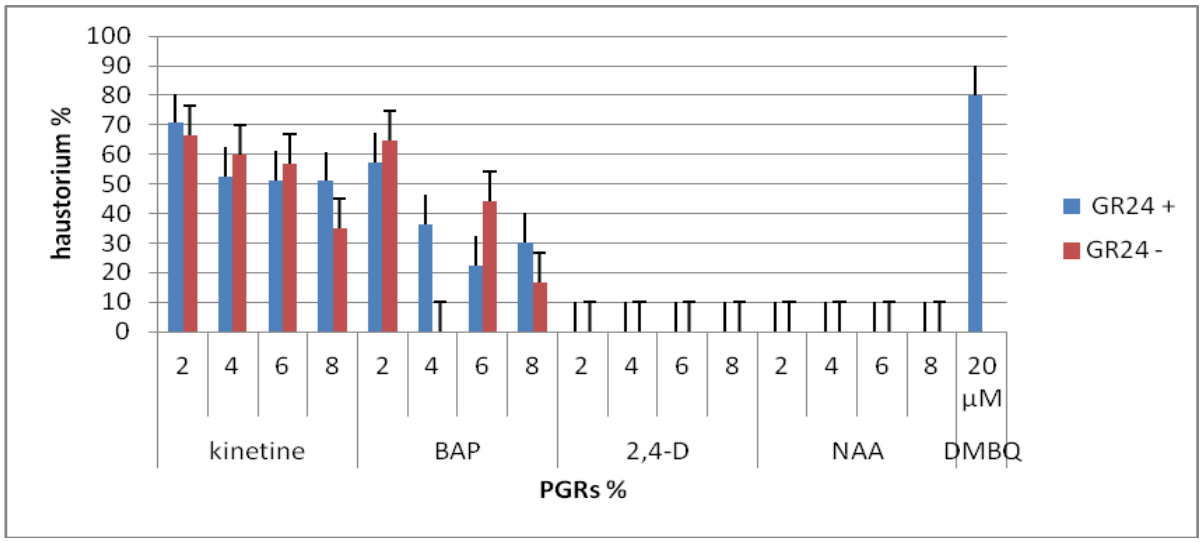

Fig. 1b. Effects of PGRs on S. hermonthica haustorium formation. DMBQ served as positive control (absence)

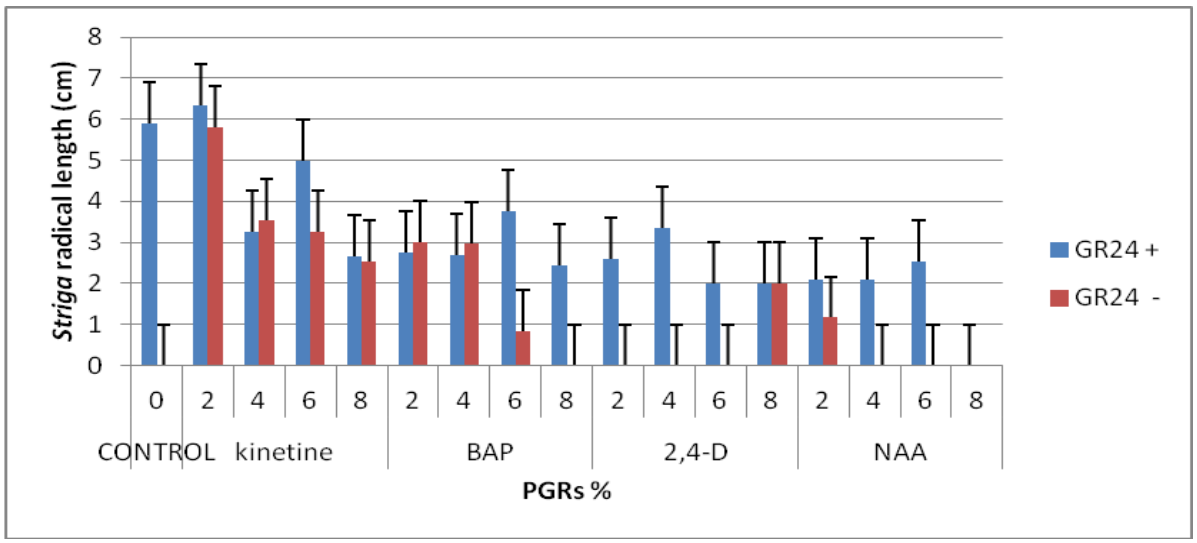

Fig. 1c. Effects of PGRs on S. hermonthica radical growth

Effects of PGRs on S. hermonthica haustorium initiation

Striga germlings arising from seeds conditioned in distilled water subsequent to GR24 treatment showed considerable haustorium initiation (88%) in response to DMBQ. In among all treatments, increase in concentration of all PGRs reduced haustorium considerably. However, in germilings treated with cytokinines kinetine and BAP, irrespective of concentrations, haustorial initiation ranged from 29-67% comparable to the control (88%) (Fig 2). A conditioned seeds in water and similarly treated with auxin 2, 4-D completely suppressed Striga haustorium (100%) in response to DMBQ as compared to the control. Auxin NAA reduced haustorium significantly and the reduction progressively increased with increased concentration (Fig 2).

Effects of the combination of PGRs and NaCl on seed germination

Seeds conditioned in distilled water and treated with GR24 at 0.1ppm induced germination to 98.8% (Fig.3a). Results displayed that all treatments reduced *Striga* seeds germination as compared to control in a dose dependent manner. Seeds conditioned in water and treated with auxins NAA or 2, 4-D in combination with NaCl reduced *Striga* germination by 90 to 100%, in response to GR24, irrespective to concentrations of auxins and NaCl.

Generally, conditioned seeds treated with NaCl had reduced germination and the reduction progressively increased with increasing concentration of the salt, except in kinetine. It increased *Striga* germination by 78 to 85% with increasing NaCl at 3.3 to 6.3 µM, respectively in presence of GR24 at the low concentration of auxin . Furthermore, auxin BAP sustained the highest germination as compared to kinetine in combination with NaCl (Fig 3a).

Striga germlings arising from seeds conditioned in distilled water subsequent to GR24 treatment showed no haustorium in absence of DMBQ (Fig.3b). However, conditioned seeds in kinetine or BAP irrespective to their concentration, combined with salt induced haustorium initiation in absence of haustorium factors (DMBQ). Nevertheless, both auxins (NAA and 2, 4-D) combined with NaCl, completely inhibited haustorum initiation.

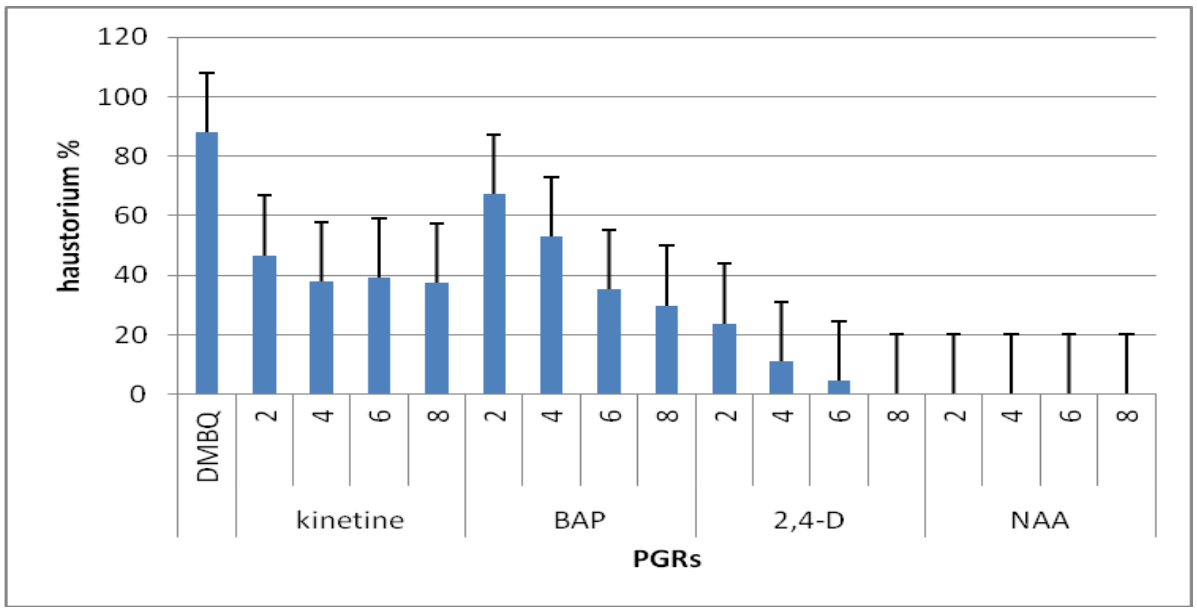

Fig. 2. Effects PGRs on haustorium iniatioation percentage of S. hermonthica in response to DMBQ

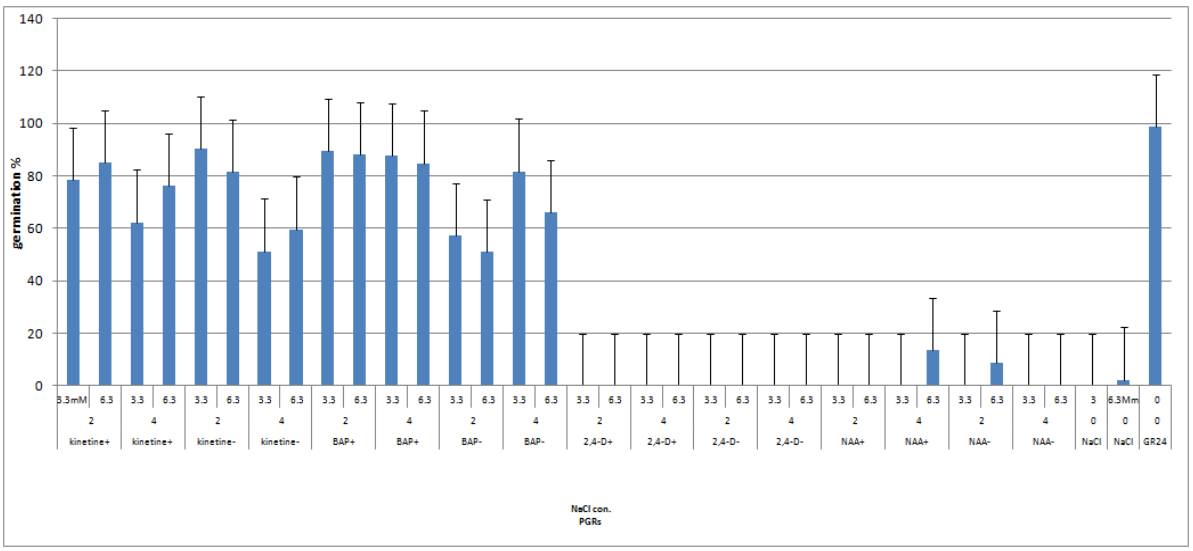

Fig 3a. Effects of PGRs and NaCl on *Striga* germination in response to GR24.

Effects of PGRs, applied during conditioning, on germination in response to GR24

Seeds conditioned in distilled water and treated with GR24 at 0.1 ppm displayed the highest *Striga* seeds germination (90%) (Fig 4). Results showed that all

treatments reduced *Striga* seeds germination, irrespective to their concentrations. In among all treatments kinetine at the highest concentration was complete inhibited germination in response to GR24. While at the lowest concentration, it reduced germination by 90% and the reduction progressively increased with decreasing GR24 concentrations as compared to the control. Conditioning Striga seeds in auxins NAA or 2, 4-D reduced germination (47 - 100%) as compared to water control and the reduction progressively increased with increased concentration of auxins irrespective to GR24 concentrations.

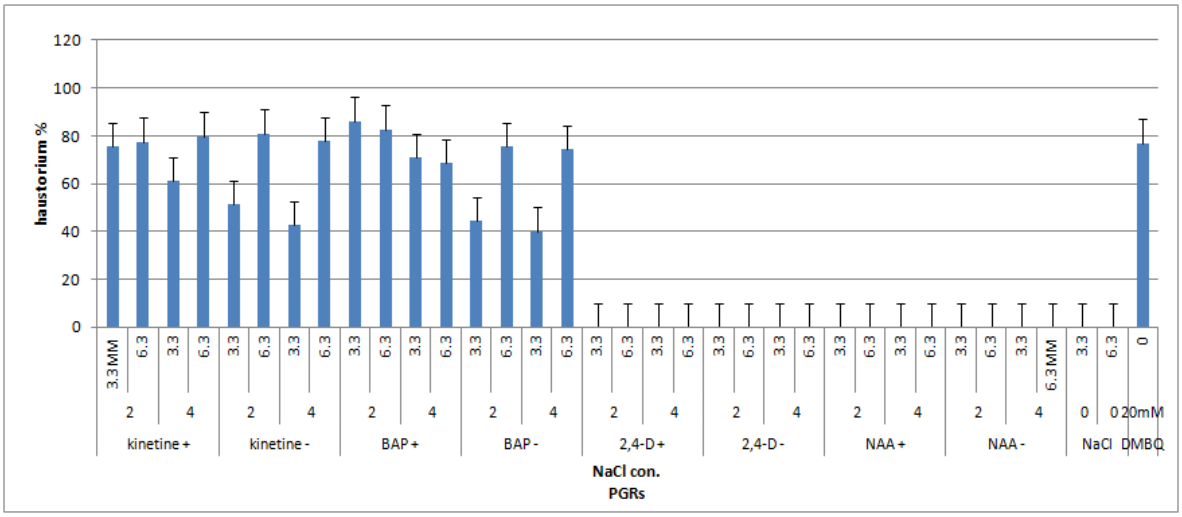

Fig 3b. Effects of PGRs and NaCl on *Striga* haustorium initiation in absence of DMBQ. DMBQ served as positive control. + with, _ without

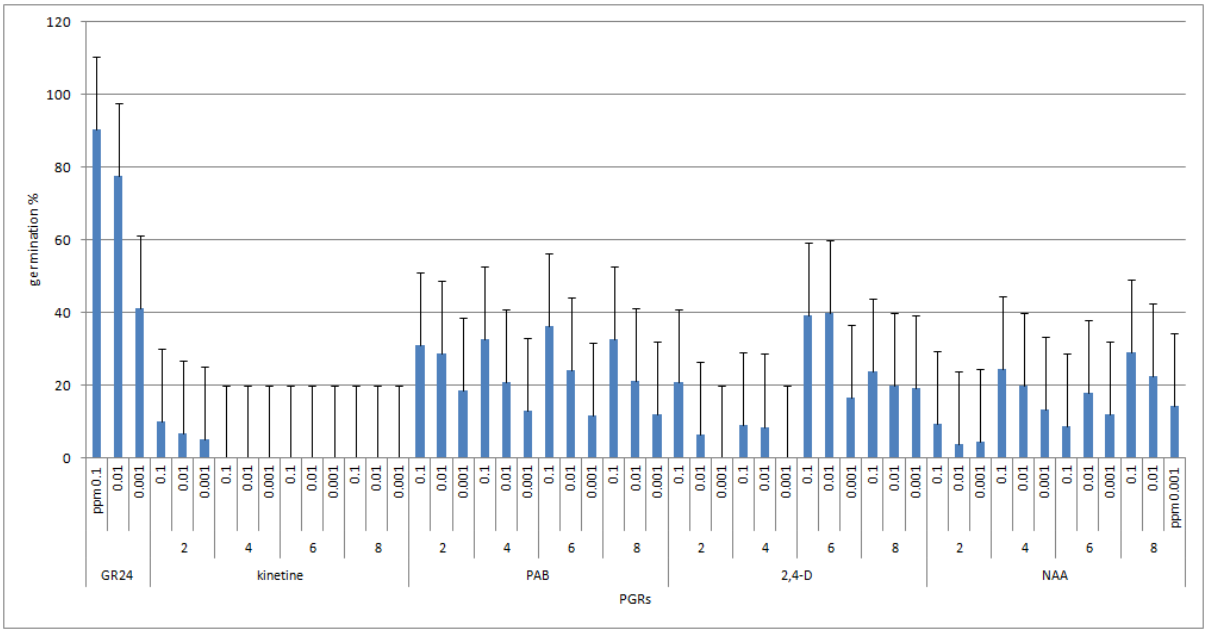

Fig 4. Effects PGRs, applied during conditioning on germination percentage of *S. hermonthica* in response to the synthetic germination stimulants GR24

Discussion

Witchweed seedlings are dependent on a host plant not only for their nutrition, but also for plant hormones such as acytokinine required for the promotion of shoot development. Auxin negatively regulates Cytokinine biosynthesis to control some

plant developmental stages (Nordstrom *et al.*, 2004).The present study displayed that Auxin-applied simultuneously with strigol analouge completely abolished germination. Exogenous cytokinines kinetine and BAP in response to GR24 induced maximal germination of *Striga hermonthica*. *Striga* seed previously conditioned in water and subsequently treated with BAP, induced both germination and haustorium initiation. However, BAP applied to *Striga* seed during conditioning, reduced germination in response to GR24. This finding is in line with previous reports of Ibrahim *et al.*, (1985). Germination of conditioned Striga seeds is known to be triggered by several synthetic and natural compounds which are structurally unrelated (Worsham, 1987). The reduced response of *Striga* seeds to GR24, applied subsequent to conditioned seeds in water and treated with auxin consistent with numerous reports (Vallance, 1951). Striga seed treated with germination stimulants, irrespective of conditioning, was reported to display considerable increase in respiration (Vallance, 1951). Several authors reported that prolonged exposure of seeds to CO_2 may reduce activity and/or synthesis of ACC synthase (Babiker *et al.,* 2000; Mathooko, 2001). Hassan *et al.*, (2011) reported that Both 2, 4-D and NAA auxins, irrespective of concentrations level displayed no *Striga* seeds germination. However, IAA and IBA displayed 100% germination as compared to the control. Cytokinines (kin and BAP) induced Striga seeds germination.

Plant hormones are probably also necessary for the haustoria formation, which is the next specific developmental stage of parasitic plants from the genus Striga. Exogenous cytokinins induce haustorial development in *Striga asiatica*, whereas auxins strongly inhibit both cytokinin and bensoquinone induction (Keyes *et al.*, 2000). Induction of haustorium initiation by cytokinine is in line with the previous finding of Ibrahim *et al.* (1985) that cytokinines (kinetine and BAP) induced haustorium initiation in Striga hermonthica. Haustorium-initiating substances consisting of phenolic compounds and cytokinins, among others, are widely distributed in plants (Parker and Riches, 1993). Haustorium initiation concurrent with germination results in perturbation of the orderly sequence of events in *Striga* germination and development. It leads to shortening of radical and hence minimizes contact with the host roots. Furthermore, haustoria are non-specific in attachment and may even attach to soil grains or glass surfaces (Tomilov *et al.*, 2008). *Striga* haustoria penetrate host roots through chemical or enzymatic means rather than by physical pressure. Moreover, S. hermonthica is influenced by exogenous growth regulators (Keyes *et al.*, 2001). However, the instability of most, if not all germination stimulants known to-date, coupled with variability of conditioning status of the parasite seeds within the soil profile put serious limitations to the practical implementation of the concept (Kgosi *et al.*, 2012).biothynthetic. This approach, if succssful would increase the efficiency of available control measures and might have a major impact on weed control at large. Babiker *et al* (1993) also observed that cytokinins increase the capacity of *S.asiatica* seed to convert ACC to ethylene and to increase the germination of seeds. Based on previous reports that cytokinins enhance ACC oxidase activity in plant seeds and vegetative parts and that auxins have synergistic effect on ethylene production and regulation in several plants (Fellman *et al* ., 1987), Babiker *et al.* (1994),demonstrated the cytokinin-like activity of thidiazuron, which when combined with several auxins elicits ethylene production. It was concluded that ethylene biosynthesis and action are crucial or essential in the germination of *Striga*; and that dormancy in *Striga* seeds is associated with the low capacity of the seeds to convert ACC to ethylene. Furthermore, Osmotic potential may significantly affect germination and haustorium initiation of *Striga hermonthica*.

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