CHAPTER 4

THEY STAND AMONG EQUALS:
III. SEED GERMINATION AND GROWTH PATTERNS OF NEW BIOTYPES OF WEEDY RICE (*Oryza sativa* L.) IN SELANGOR NORTH-WEST PROJECT, MALAYSIA

4.1 INTRODUCTION

Higher plant seeds go into a phase of developmental arrest, otherwise known as primary dormancy, before reaching the maturity (Gardner *et al.* 1985). For germination to occur, inactive or non-dormant seeds require only some water following the release from primary dormancy while dormant seeds need additional external stimuli such as light, temperature and chemicals in order to be released from their dormant state (Thomas 1992; Mancinelli *et al.* 1967; Small & Gutterman, 1991). In many wild plant species, seeds in a given population do not germinate at the same time under a given set of conditions favourable for seedling growth (Frankland & Taylorson 1983). When allowed to imbibe, some seeds can germinate in the dark, whereas others even in the same population fail to germinate and require some physical or chemical stimuli or both such as light, temperature and chemicals. Among these environmentally-controlling stimuli, light is the best known and characterized factor (Gardner *et al.* 1985).

Weedy, wild or red rice are weeds that are highly related to cultivated rice but generally known has greater primary dormancy, more tillers, longer culms, high susceptibility to seed shattering, pubescent leaves and red pigmentation of pericarp, seed coat, or both (Diarra *et al.* 1985; Suh *et al*. 1997; Noldin *et al.* 1999). It is well known in rice and any other cereals crops; light irradiation is not generally required for germination of wild, weedy, or any other cultivated rice seeds (Chung & Paek 2003).

Many researchers have reported that seasonal variations in daily maximum and minimum temperatures stimulate seed germination, but the physiological mechanisms have not been clarified because the optimum ranges of diurnal temperatures and fluctuation gaps are quite different among the species. Buried seeds receive attenuated amounts of heat and light according to burial depth and transmissivity of the soil (Simpson 1990; Benvenuti *et al*. 2001).

Studies on seed dormancy is important in establishing weed populations as it allows seeds to germinate, hence avoiding poor conditions that may prevent seedling to grow at different times of the growing season. Seed dormancy combined with longevity enables the persistence of seed bank to grow in the soil. Seed dormancy is generally weaker in cultivated rice than in weedy rice (Oka 1988; Vaughan 1994). Unfortunately, the longevity of rice seeds has not been well studied but some studies show wild rice seeds were shown to be more venerable (Vaughan 1994), and can be dormant for several years (Moldenhauer & Gibbons 2003).

In any growing crops, dormancy of weeds are vital and has been considered importance to pre-harvest emergent and the survival of weed seeds in the soil. Pre-harvest emergence is germination in the inflorescence after maturation of the crop, when the environment is suitable for the seeds to emerge especially in existence of moist or rain. Resistance to pre-harvest emergent is influenced by the level of dormancy in dry and seeds maturity (Seshu & Sorrells 1986). Dormancy is usually time-mediated, and is critical for the germination of weedy plants.

Simpson (1990) defined dormancy as the incapability of a viable seed to germinate temporary after a specific length of time, especially in restrictive environmental conditions that not allow germination of seeds by either natural or artificial conditions (Simpson, 1990). Despite years of research on seed dormancy, mechanisms for the regulation of germinability are basically unknown (Foley 2001; Koornneef *et al.* 2002).

Germinability is used to describe the capacity of seeds in a population with dormancy for immediate, intermediate, or much delayed germination due to the internal conditions (Foley, 2001). Seed longevity in the soil or seed bank is an additional character that allows population persistence over cropping seasons for the weedy rice. Contaminated rice seeds used in rice farms and movement of machinery and tools between granaries are also factors related to this problem. Moreover, the natural seed dormancy in some accessions of weedy rice makes control and management more difficult in rice cultivation. For that reason, holistic control and management have to be developed which integrate indirect control such as thorough land preparation, high quality seeds, and appropriate seeding rate and crop establishment technique; with direct control such as pre-emergence and pre-planting herbicides, manual weeding and rouging (Baki *et al.* 2000).

Weedy rice seeds show a variable degree of dormancy contrasting with cultivated varieties. The duration of the dormancy differs according to the accessions and the storage conditions of the seeds after shattering. The dormancy length has been studied in several countries in natural conditions. For example, feasible weedy rice seeds with red pericarp, stayed dormant for up to two years in the United States (Klosterboer 1978) and three years in Brazil. In the other hand, *Oryza punctata* was dormant for more than one year in Swaziland (Armstrong 1968) and in East Africa, the can survive to five years (Majisu 1970).

Seed longevity also has been examined in several other studies. In a research in the United States, seeds from different populations of weedy rice remained vital by 90 % after two years of burial and up to 20 % after seven years. Diarra *et al.* (1985) suggested that the longevity of the weedy rice seeds can last for up to 12 years.

In a study conducted in Italy the viability of weedy rice seeds taken at a depth by ploughing in loamy soil decreased to 6% after one year and to 5 % after two years of burial. It was assumed that most seeds can germinate under favorable conditions such as high temperature and oxygen content following tillage operations, but relatively cannot emerge from the soil. The germination percentage of the viable seeds varied in time, decreasing from 91 % at the beginning of the experiment to 73% after one or two years of burial (Ferrero & Vidotto, 1998). This behavior was showed that many of the seeds that were dormant at the beginning of the experiment did not germinate and remained dormant for over two years. The seeds dug up after one year required less time to germinate than those buried for two years.

Environmental conditions during seed formation, moisture and the storage temperature are considered to be the main factors that can affect the length of dormancy (Ferrero 2003). Leopold *et al.* (1988) reported that the weedy rice seeds of the straw colour pericarp accessions, showed a variable duration of the dormancy in relation to the humidity content of the seeds after ripening even after kept at -15°C. The breaking of dormancy was quicker at grain humidity ranging from 6-14 % and very low at water content lower than 5 % or higher than 18 %.

Dormancy reduction significantly occurs two months after ripening (Cohn & Hughes 1981). Even though a number of studies have been done at a biochemical level however to define the physiological and genetic bases of dormancy using the mechanisms of the inception and breaking of this phenomenon have not yet been completely clarified (Cohn 1996; Cai & Morishima 2000). Dormancy regulation can most likely be recognized through the factors present in glumella and in embryo (Ferrero and Vidoto 1998).

There are two common ways seed dormancy is imposed which is the seed coverings for example the pericarp, testa and in some cases the endosperm and also is imposed by the embryo itself (Bewley & Black 1994). In grasses, the term seed is commonly used to describe the dispersal unit (Simpson 1990). There is a plethora of evidences that seed dormancy in rice is imposed through the hull, pericarp or testa, or both and an intact rice seed has a hard enclosure-hull outside the caryopsis. (Seshu & Sorreles 1986; Gu *et al.* 2003). The occurrence of embryo dormancy in rice has been suggested but remains uncertain because some reports indicate that rice lacks embryo dormancy (Seshu & Dadlani 1991). This character of dormancy in rice can be related with weedy rice as they share almost the same characteristic morphologically with cultivated rice.

Weedy rice usually goes together with the cultivation of rice in world's rice industry. Generally, seed dormancy is stronger in weedy rice than in cultivated rice (Oka, 1988; Cho *et al*. 1995; Suh *et al.* 1997; Tang & Morishima 1997). It is likely that weedy rice has novel alleles that influence germinability and are not present in domesticated rice (Gu *et al.* 2006).

The work embodied in this chapter will discuss on seed germination and growth pattern of new biotypes of weedy rice. The experiments were conducted to test various environment effects on NBWR's seed germination. The results from these experiments hopefully can give us an insight and brief understanding on how NBWR can survive as influenced by environmental variables. Furthermore, seed dormancy in NBWR, if any, can also be assessed as well.

4.2 MATERIALS AND METHODS

4.2.1 Seed Production

The NBWR plants described in Chapter 3 were chosen for this experiment. A plastic bag was placed to every panicle of NBWR during the maturity of the plant to collect the seeds produced by NBWR. The harvested seeds were then collected and the panicle length was measured. After that the seeds were removed from the panicle. The collected seeds of each panicle were weighed and counted. An average estimation of seed production was calculated between panicle length and number of filled grain/panicle.

4.2.2 Seed Weight

One thousand seeds were randomly selected from each plant as described in Chapter 3, and were weighed using a Mettler Toledo Model ab204 autobalance. Four group weights were formed to differentiate the seed groups by weight. For every weight group, 100 seeds were sown to assess germination rate as influenced by seed weight.

4.2.3 Time of Shattering

The time of seed shattering of NBWR plants described in Chapter 3 were observed. Days after sowing (DAS) will be recorded when more than 50% seeds in the panicle start to shatter by grasping it gently and the shattered seeds were carefully collected in paper bags.

4.2.4 Plant materials

NBWR's seeds were harvested at maturity from Tanjung Karang to Bagan Terap granaries in 2006-2007. All seeds were then air-dried to remove any moisture and then were kept in room temperature before use.

4.2.5 Germination Test

Tests on germination were conducted in a 14.5 diameter petri-dishes lined with one layer of moist Whatman no. 44 filter paper. For every petri dish, 10 seeds were sown. A total of 32 petri dishes were used in this experiment. Distilled water was added to the petri dishes everyday to keep the seeds moist and the petri-dishes were kept saturated. Germination was recorded everyday for 20 days. The number of germinated seeds was counted and the percentage of germination was calculated.

4.2.6 Temperature effects

The germination test was conducted in a growth chamber which was set up at different temperature levels to determine the optimum temperature for the germination of

NBWR's seeds. The temperature gradient used was between 25°C and 45°C for 20 days and monitored for germination. Four replicates were used for each temperature (25°C, 30°C, 35°C, 40°C and 45°C) with 20 seeds per replicate. Germination was recorded every three days and germination percentage was determined after 20 days after seeds were sown. Seeds were considered to have germinated when the radical has emerged for 5mm or more.

4.2.7 Light Requirement

Seeds were germinated in two light regimes; 12 hours of exposure to light alternated with 12 hours dark (12L - 12D), and a regime with constant darkness. Twenty seeds for each four replicates for each regime were sown in petri dishes. Those in constant darkness were covered with aluminum foil to prevent any exposure to light. Germination was recorded every three days and germination percentage was determined after 20 days after seeds were sown. Seeds were considered to have germinated when the radical has emerged for 5mm or more.

4.2.8 Soil Depth

Ten seeds were sown into plastic pots (23cm diameter, 20cm height) filled with moist paddy soils of the Java series obtained from the rice granary of Selangor North West Project. Fifty seeds were sown per pot at the different soil depth as shown in Table 4.1 with four replicates for each depth. Pots were watered daily. Germination was recorded every three days and germination percentage was determined after 20 days after seeds were sown. Seeds were considered to have germinated when the radical has emerged for 5mm or more.

Table 4.1. Pot sown with new biotypes of weedy rice at different soil depths.

Pot Number	Depth from surface (cm)
Pot 1	0 cm (surface)
Pot 2	1 cm
Pot 3	2 cm
Pot 4	3 cm
Pot 5	4 cm
Pot 6	5 cm
Pot 7	6 cm

4.2.9 Flooding Test

Fifty seeds were sown into plastic pots (30cm diameter, 40cm height) filled with moist paddy soils with six different water levels above soil surface as shown in Table 4.2. Germination was recorded every three days and germination percentage was determined after 20 days after seeds were sown. Seeds were considered to have germinated when the radical has emerged for 5mm or more.

Table 4.2: Pot sown with new biotypes of weedy rice at different water levels.

Pot Number	Water level from soil surface (cm)
Pot 1	1 cm
Pot 2	2 cm
Pot 3	3 cm
Pot 4	4 cm
Pot 5	5 cm
Pot 6	6 cm

4.3 RESULTS AND DISCUSSION

4.3.1 Seed Production

The NBWR panicle lengths from 100 random NBWRs range from 23 cm to 28 cm with a mean length of 25.75 cm (Table 4.3). As panicle length increased (Fig 4.1), the estimated number of seeds per panicle also increased, ranging from 56 to 84 seeds per panicle with a mean of 68.29 seeds. The number of filled grains of NBWR ranged from 44

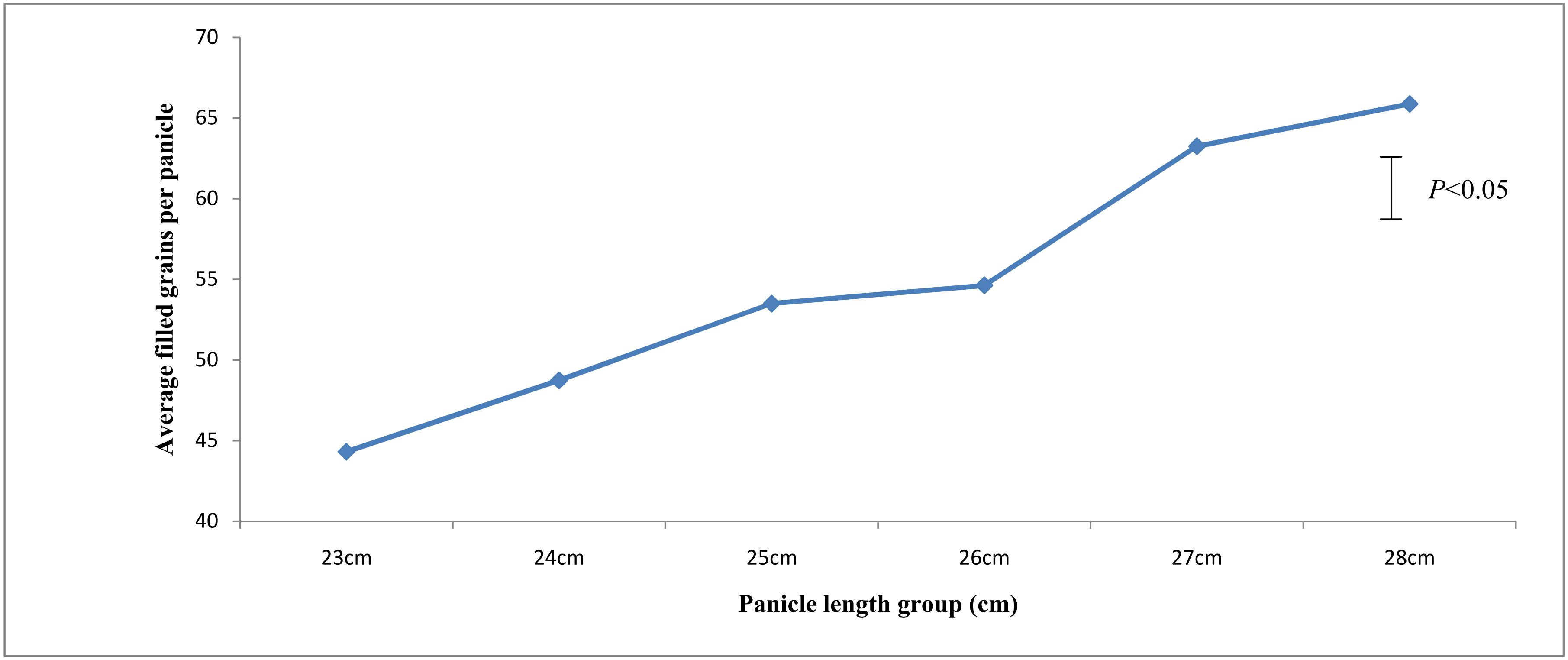

Fig. 4.1 Average filled grains per panicle according to different panicle lengths. Bar represents LSD value at $P<0.05$.

to 66 seeds per panicle (a mean of 54.63 grains/panicle). These seeds consist of *ca*. 80% of filled grain/panicle (Table 4.3).

Table 4.3: Summary of results in some germination and quantitative data test of new biotypes of weedy rice.

	Seedling height in cm (after 20DAS)	**Panicle length (cm)**	**Grain per panicle**	**Filled grain per panicle**	**1000 grains weight (g)**
Min	16.00	23.00	56.00	44.00	12.81
Max	28.00	28.00	84.00	66.00	27.77
Average	20.05	25.75	68.29	54.63	21.12

4.3.2 Seed Weight

Seeds can be visually pre-classified as filled or non-filled in an attempt to determine the probability of germination. However, weights of individual seeds also can be measured to determine whether seed weight could be correlated with germinability. One thousand grain weight has an average weight ranging from 12 g to 28 g (Fig 4.2). The average weight for NBWR seeds are 21.12 g/1000 seeds (Table 4.3). These results indicate that percentage of germination increased as seed weight increased (Fig 4.3). Seeds whose were weight greater than 20 g had significantly higher germination than those weighing less. The highest germination occurred in the 20-25 g weight group with 82.5% germination, followed by 20.25%, 60% and 48.75% in 1-15g, 15-20 g and 25-30 g group weight respectively. This result suggests that seed germinability maybe influenced by seed weight.

4.3.3 Time of Shattering

The NBWR seeds start to shatter 89 to 93 days after sowing (DAS) (Fig 4.4). From 100 NBWR plants, the average time of commencement of shattering for NBWR was after

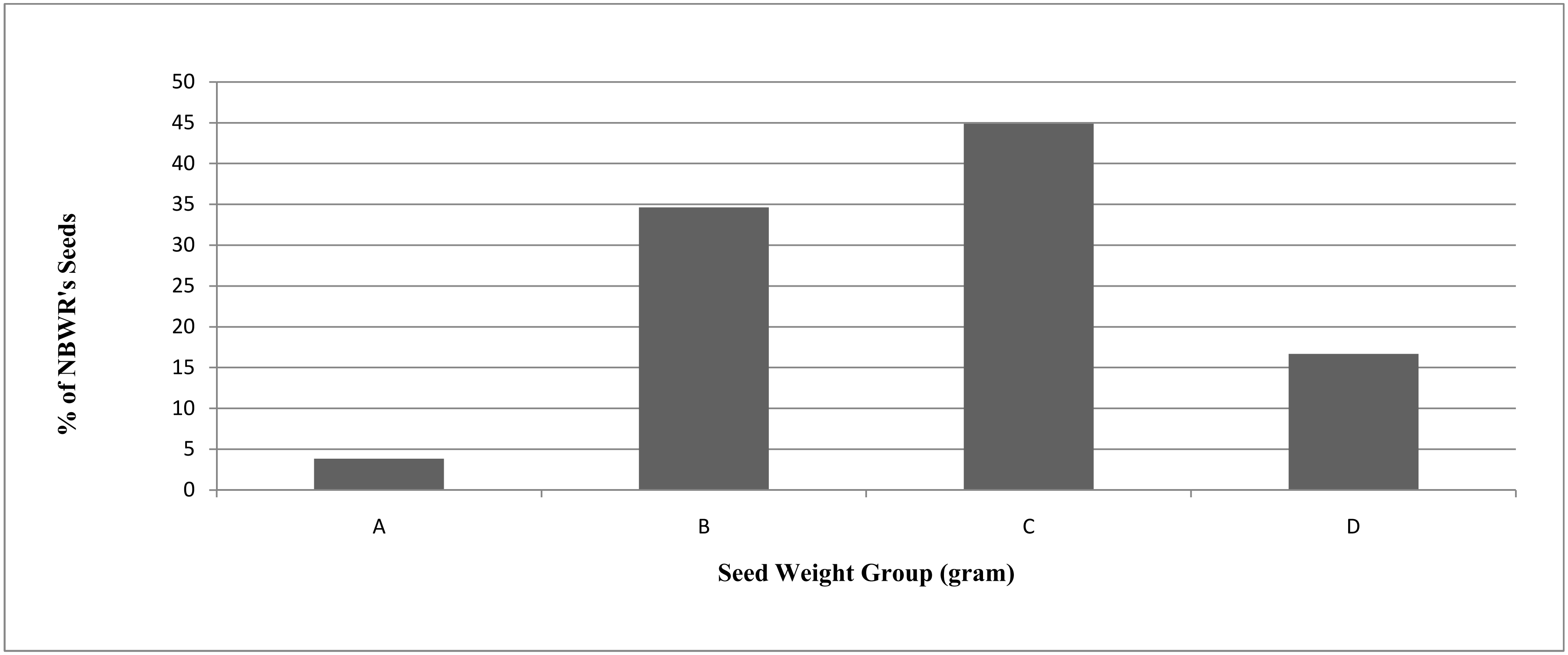

Fig. 4.2. Percentage of NBWR's seeds according to seed-weight group. Seed weight ranging from: (A) 1g-15g; (B) 15g-20g; (C) 20g-25g; (D) 25g-30g.

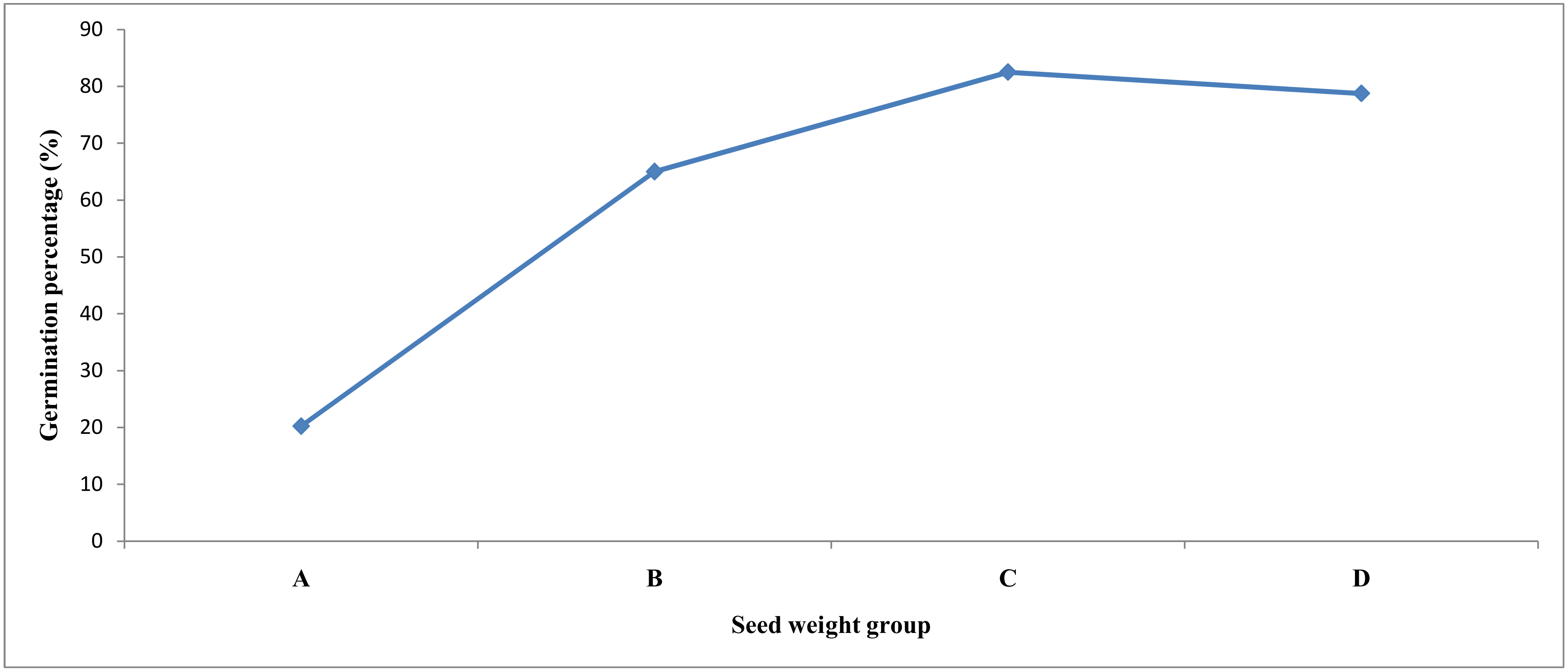

Fig. 4.3. Germination percentage according to different seed weight group. Seed weight ranging from: (A) 1g-15g; (B) 15g-20g; (C) 20g-25g; (D) 25g-30g.

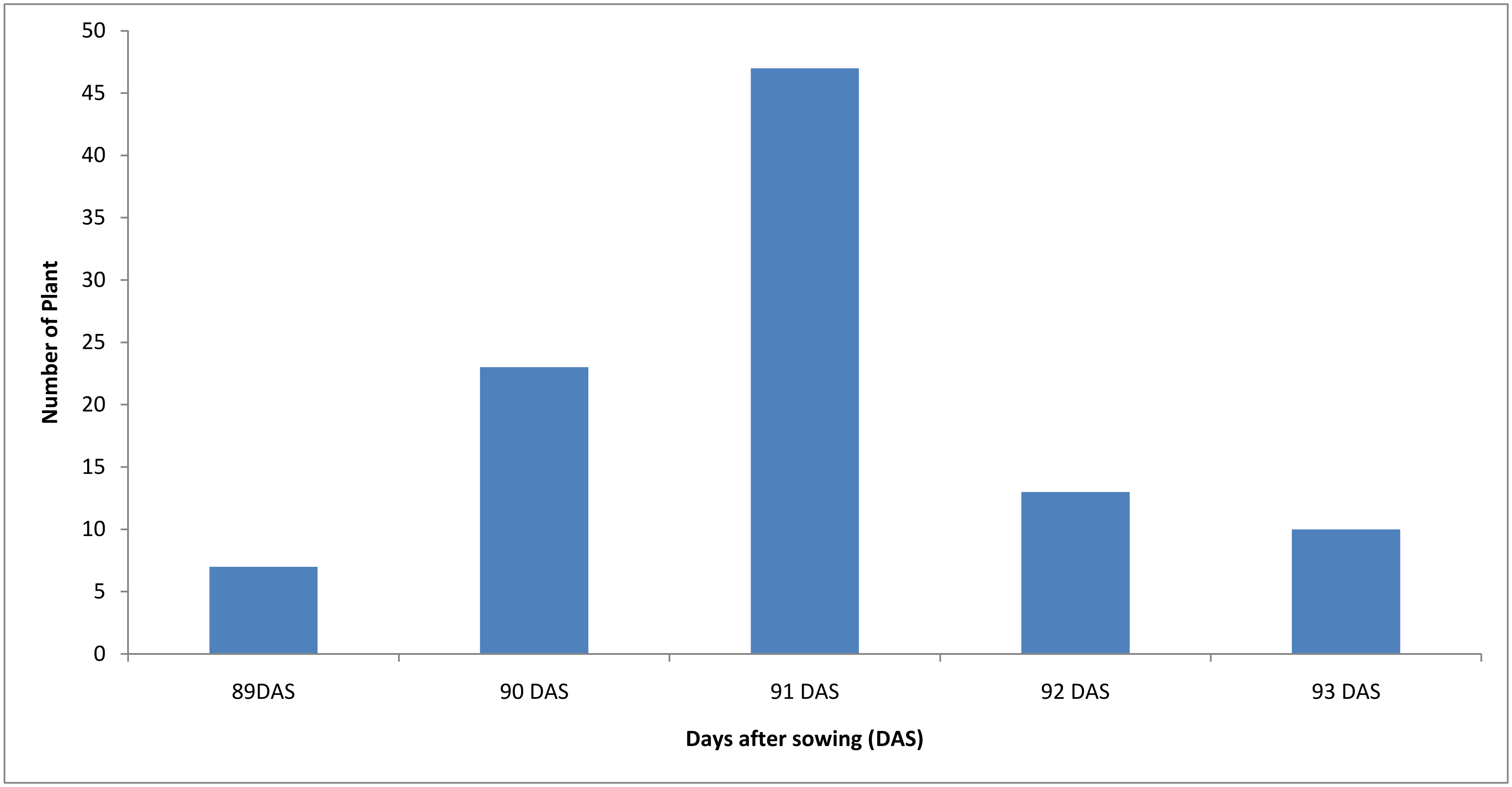

Fig. 4.4. Time of shattering for new biotype of weedy rice in Selangor North West Project.

91 DAS. Azmi *et al.* (2003) reported that most of the weedy rice (taller biotypes) starts shattering their seeds between 13 to 14 days before maturity, and shattering occurs 10 to 20 days before the maturity of cultivated rice namely MR84. MR220 has a maturity time between 105-113 DAS. In this case, the NBWR shatters between 14 days to 22 days before MR220 rice crop (the popular variety cultivated in Selangor North West Project granaries) mature.

4.3.4 Germination Test

Twenty days after sowing, there were 90.65% of total 320 seeds germinated. These results served as the control *vis-à-vis* other environmental effect for germination. Fig.4.5 and Table 4.3 show the germination pattern of the NBWR seeds. After 20days, seedlings height was ranged from 16 cm to 28 cm and 18.75% of them were 21cm in height**.** The average seedling height is 20.05 cm (Table 4.3).

4.3.5 Temperature effects

Seed dormancy can complicate management by increasing persistence of the weed seeds in the environment. With most invasive plants, weed seed dormancy can increase the time required to control a particular species and generally accounts for the requirement of a long-term follow-up in any management plan.

Under controlled conditions, temperature influenced of NBWR seed germination. Fig. 4.6 and Fig. 4.7 show the temperature gradient and its influence on the germination percentage and seedlings height. The NBWR seeds germinated at temperatures ranging between 20^{o}C and 40^{o}C, with optimal germination rate at about 30^{o}C. Germination did not differ significantly among temperatures except those exposed at 20^{o}C and 40^{o}C. However, as in plant height, NBWR seedlings seedlings exposed to 30^{o}C displayed faster growth *vis-à-vis* those subjected to other temperature regimes.

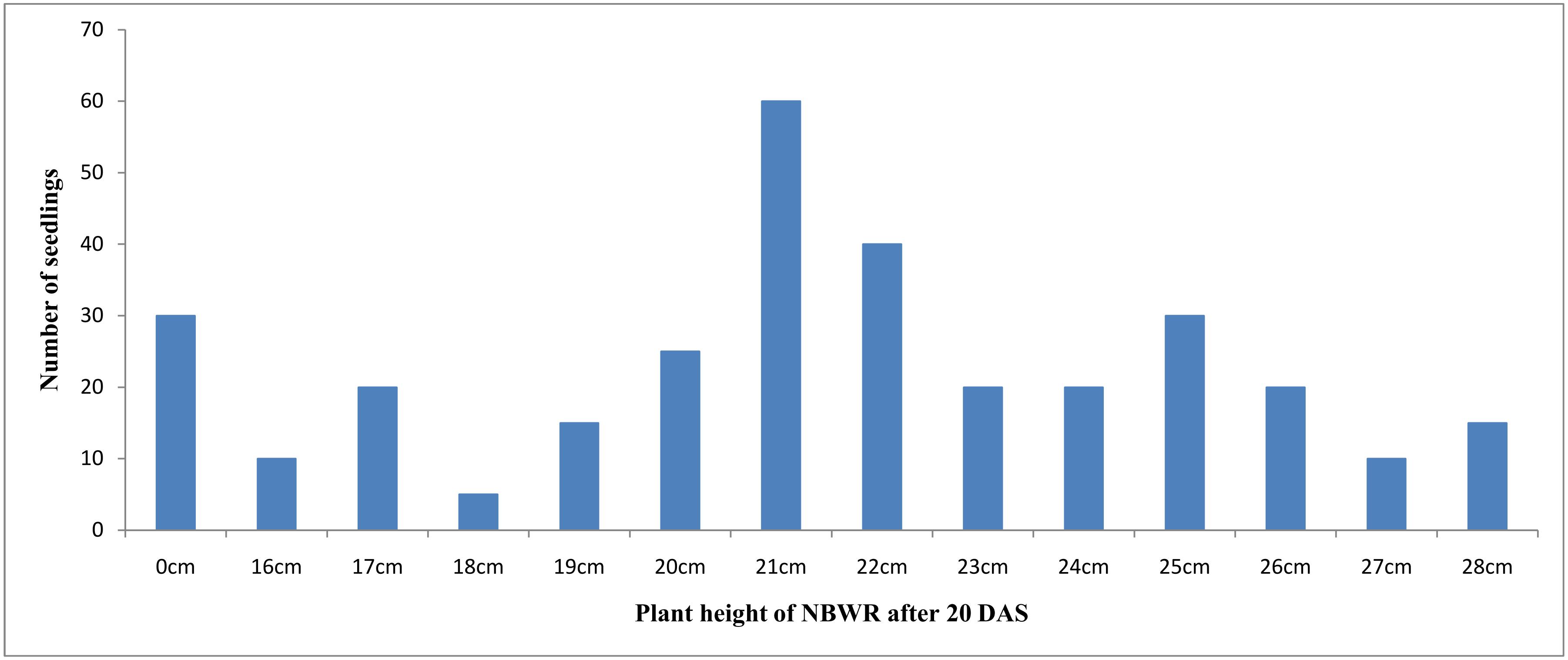

Fig. 4.5: Number of seedlings of new biotype of weedy rice (NBWR) according to different group of plant height 20 days after sowing (DAS). Bar represents LSD value at p>0.05.

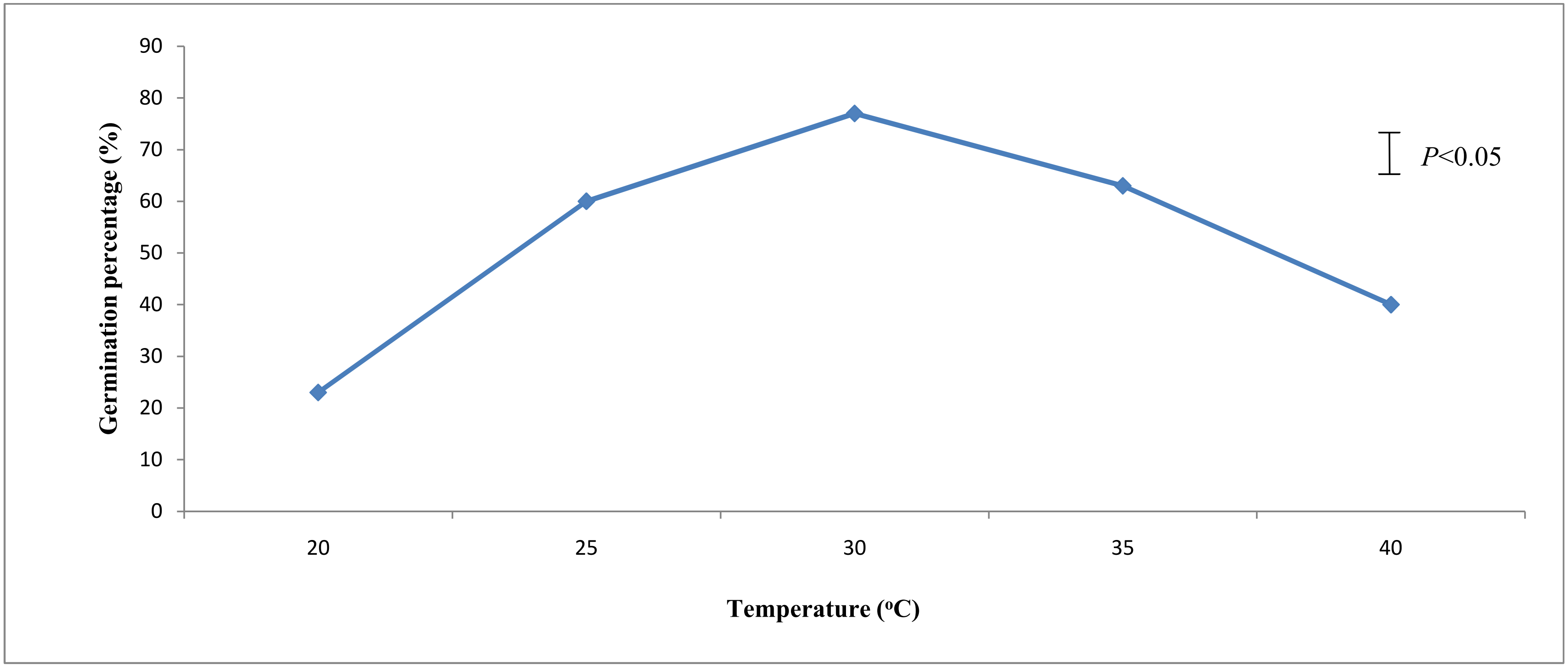

Fig. 4.6. Germination rate of NBWR in percentage according to different temperature groups. Bar represents LSD value at $p<0.05$.

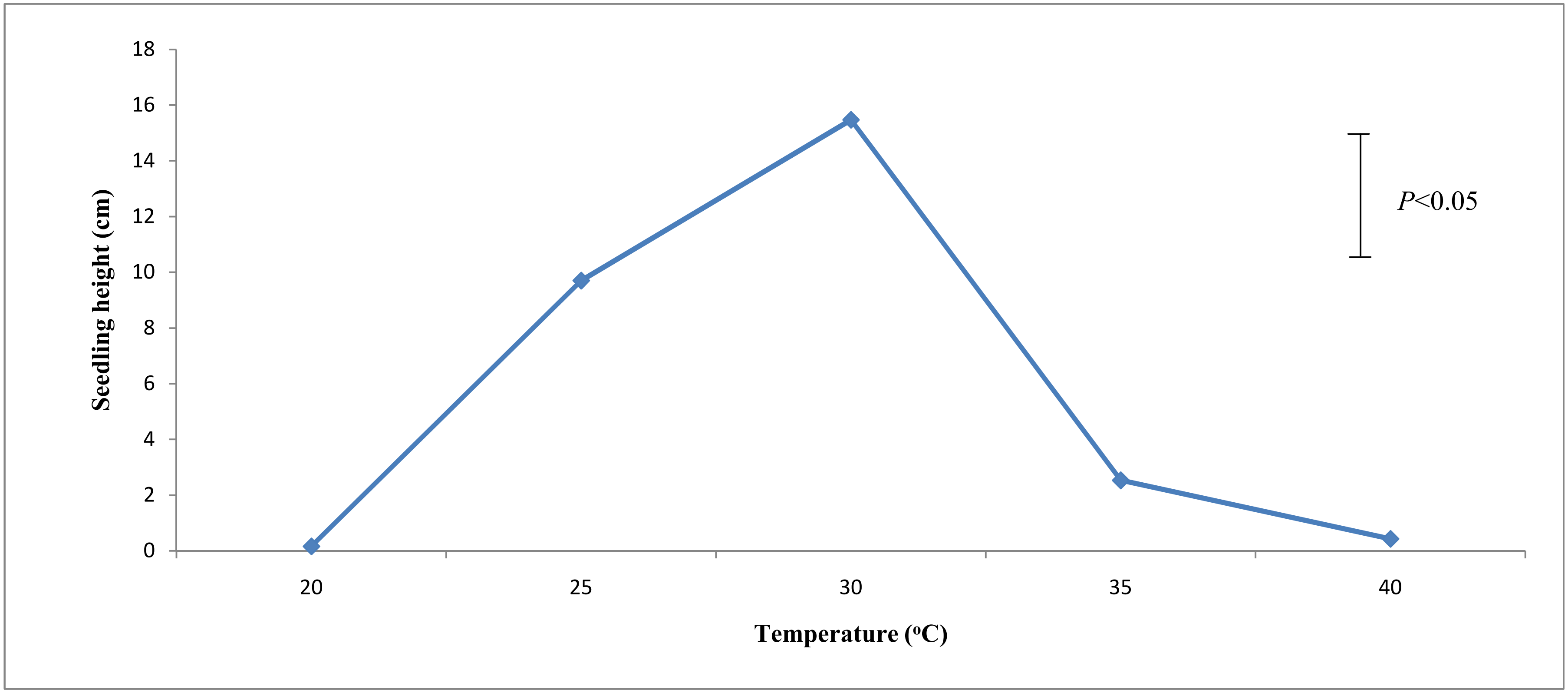

Fig. 4.7. Seedling height of NBWR 20 days after sowing when grows in different temperature regimes. Bar represents LSD value at $p<0.05$.

4.3.6 Light Requirement

Light apparently does not affect rice (*Oryza sativa*) seed germination (Chung & Paek 2003). Similar patterns were recorded in NBWR seeds. In darkness, the germination rate of NBWR was about 77%, while at 12 h-diurnal fluctuations the germination frequency was 80%. Seedlings emerging form seeds exposed to both conditions of darkness and 12h-diurnal fluctuations also have almost the same mean height 20 days after sowing which are 17.37 cm and 17.93 cm, respectively.

4.3.7 Soil Depth

Seedling emergence frequency decreased with burial depth (Fig. 4.8). At the surface (0 cm), almost 80% of seedlings emerged. At 1 cm depth, 73.33% of the seeds germinated and this was followed by 63.33% at 2 cm depth, 40.00% at 3 cm depth, 23.33% at 4 cm depth and no germination was registered at the 5 cm depth.

Seedling heights of NBWR seeds emerging from those buried at different soil depths differed. The average seedling height of those seeds at the surface after 20 days was 17.93 cm, and the heights decreased sown at the deeper soil depths (Fig. 4.9). The average seedling height of seeds kept at the 1 cm depth was 14.67 cm, 2.48 cm at the 2cm depth, 0.43 cm at the 3 cm depth and 0.17 cm at the 4 cm depth. Watanabe (1996) reported that weedy rice emerged earlier from the seed bank than the cultivated rice seeds in direct seeded conditions, and in that way gained a competitive advantage over the cultivated rice.

In many species, germination rates decrease at greater soil depths (Eastin 1983; Ayeni *et al.* 1997). Similar patterns of germination also prevailed in NBWR seeds. Data from depth experiment demonstrated a significant decrease in NBWR germination with the increase of the soil depths. The average germination of NBWR seeds placed on the surface was 80%, and no germination occurred at depths deeper than 5cm depth. It was also a possibility that more seeds germinated in the soil but exhausted their energy reserves

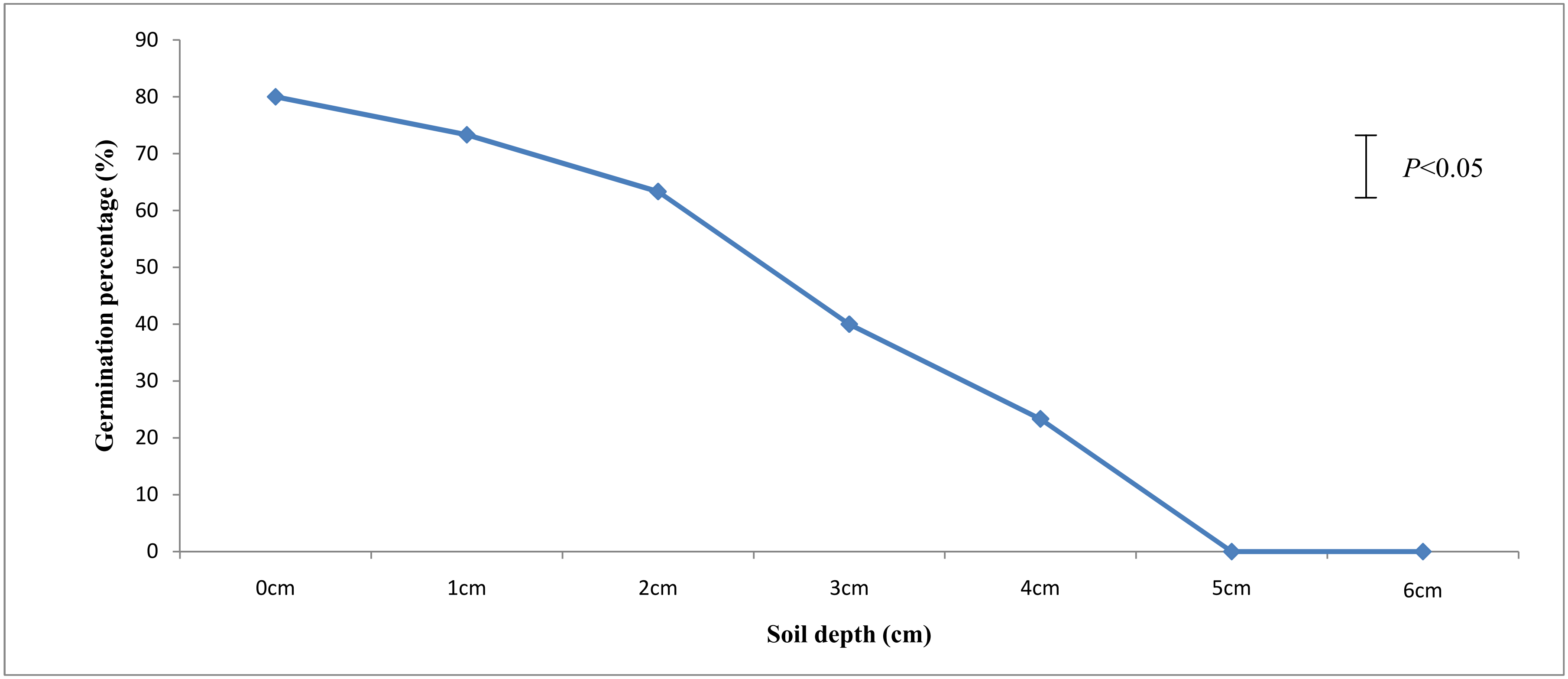

Fig. 4.8. Germination rate of NBWR in percentage according to different soil depths. Bar represents LSD value at $p<0.05$.

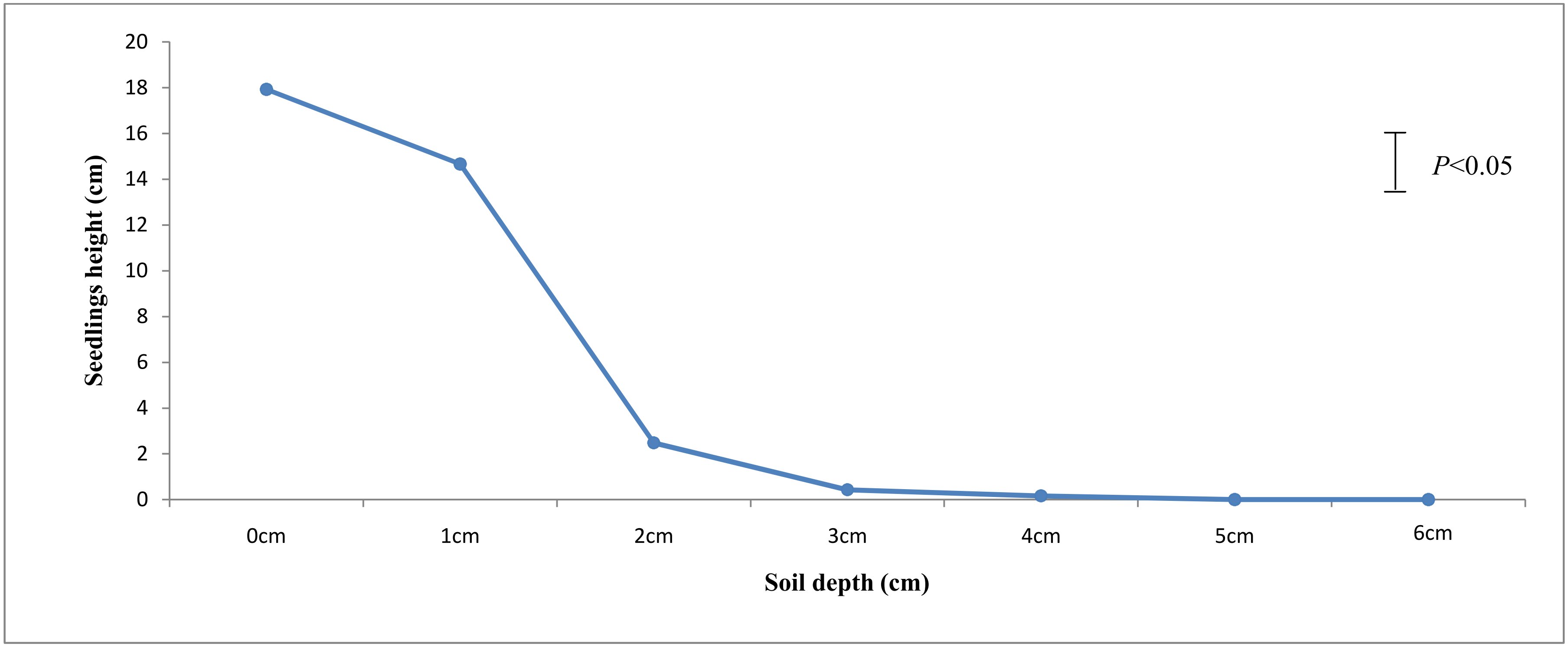

Fig. 4.9. Seedling height of NBWR 20 days after sowing when grows in different soil depths. Bar represents LSD value at $p<0.05$.

before reaching the soil surface. This pattern can be seen in Fig. 4.9 where there was a difference in plant heights of NBWR seedlings emerging from different soil depths.

4.3.8 Flooding Test

In the flooding test, the results showed measurable decrease in germination rate at different levels of inundation. More than 70% of the seeds germinated under 1 cm inundation, and the percentage of seedling germination decrease under deeper inundation (Fig. 4.10 and Fig. 4.11). No germination was recorded under 7 cm submergence. As 70% of NBWRs seeds germinated at 0 – 1 cm below the water surface, 50% of seeds germinated below 3 cm, and 27% at 5 cm inundation.

The average heights of seedlings showed a measurable decrease between 1 cm sand at 3 cm submergence. Below 1 cm, the average height of seedlings subjected to flooding was 13.27cm, while below 3 cm water depth was 1.5cm in height and 0.45 cm in 5cm submergence.

The NBWR can be controlled by applying a wet seeding method in rice granaries. Even though this contention has not been tested in the actual field conditions, but in the controlled environmental test, the NBWR seed germination and seedling growth were influenced by water level. On the other hand, if water level is kept around 5cm or 7cm above ground, NBWR seeds cannot be germinated.

4.3.9 Factors Affecting Germination and Emergence of NBWR

Several environmental factors are known to promote or inhibit weed seed germination (Burke *et al.* 2003). Temperature and light requirements for germination varied from species to species (Zhou *et al.* 2005) while certain depth in soil and water will also affect the germination of some species (Norsworthy and Oliveira 2007).

This study revealed three principal characteristics of NBWR seeds which could be of great value a far as the depletion of the soil seed bank of the rice farms was concerned

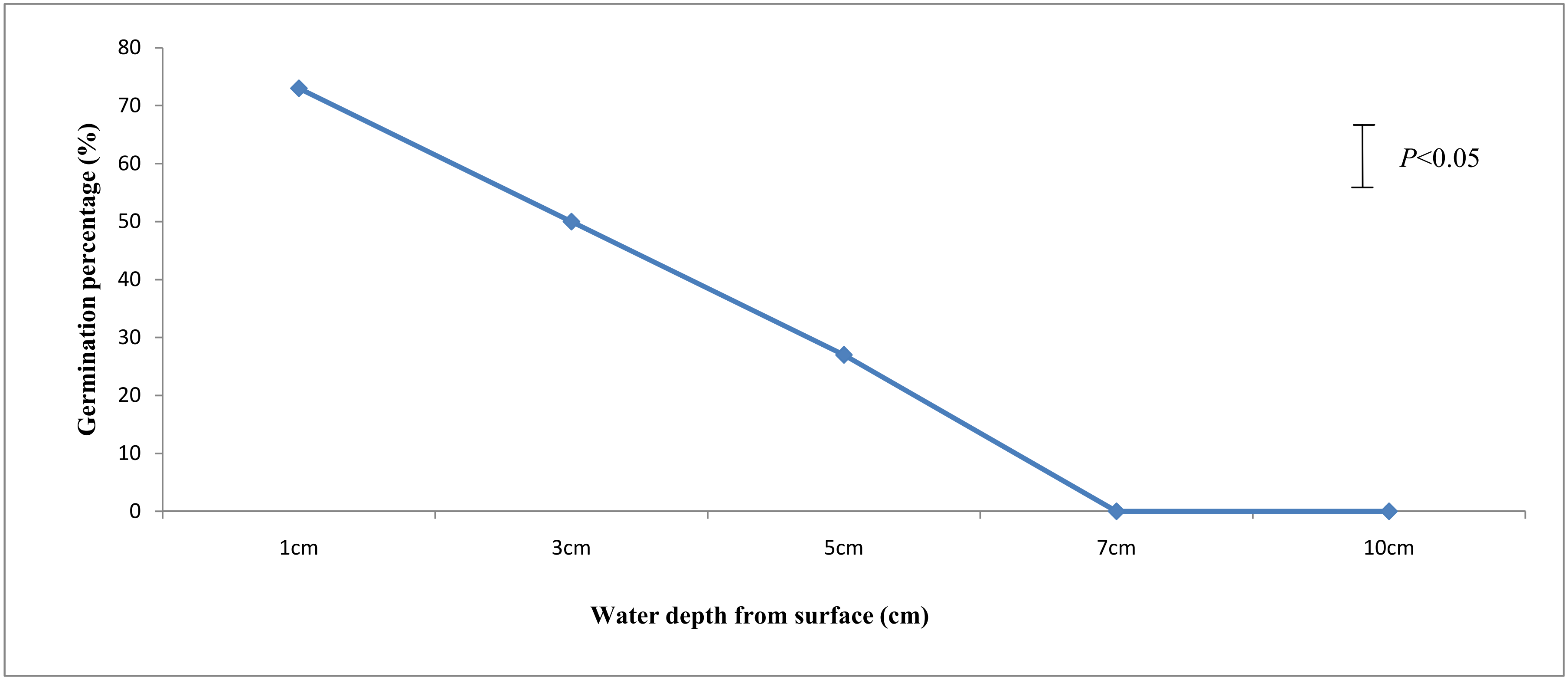

Fig. 4.10. Germination rate of NBWR in when submerged in different water depths. Bar represents LSD value at $p<0.05$.

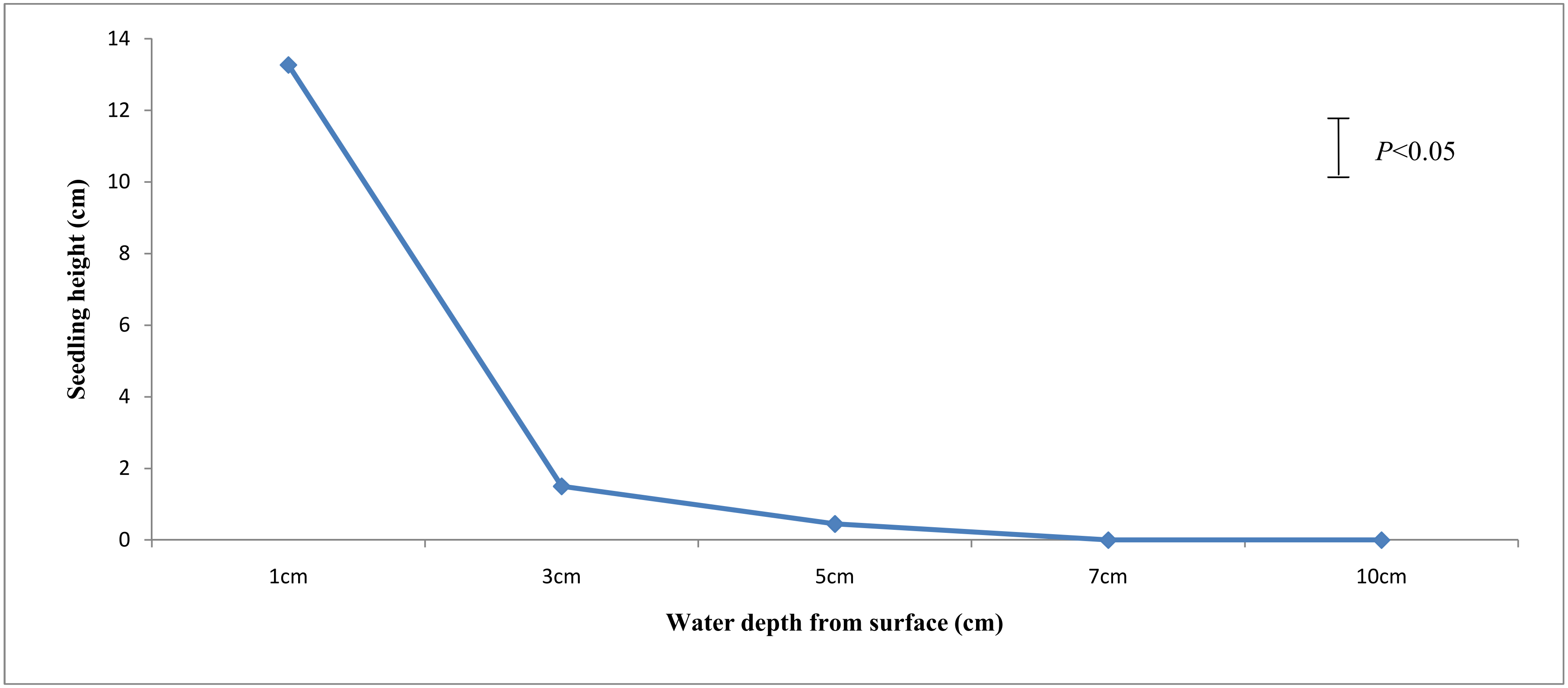

Fig. 4.11. Seedling height of NBWR 20 days after sowing when grown in different water depths. Bar represents LSD value at $p<0.05$.

viz. (i) NBWR seed needs no specific light-requirements for germination which enable the NBWR seeds to germinate in any particular light intensity (light or dark), (ii) NBWR seeds have a wide range temperature regimes to germinate, similar with any cultivated rice. This can give an indication that NBWR can grow in any place which paddy can grow and, (iii), the enhancement of germination of NBWR seeds deep below the soil surface could lead to the dormancy of the subsequent seedlings, thus in turn contribute to the depletion of the NBWR seed bank in rice farms.

Seed burial in soils inundated with water at any depth arguably, inhibited NBWR seed germination but there is no explanation yet about this situation because NBWR seeds can germinate in the dark and there is no experiment to test and to prove why NBWR seeds cannot germinate under inundated soil and water. This can be a starting point for further research on the dorminancy of NBWR seeds. From the series of experiment, NBWR seeds seemed to have no special requirements for germination.

The reason for inducing enforced seed dormancy could be attributed to seed buoyancy or the ability of NBWR seeds to germinate on the water surface rather than inundated or flood water and become established from shallow water depths during alternating periods of irrigation and field saturation. These germination characteristics probably explain the rapid encroachment and dominance of NBWR in irrigated wet-seeded rice and rain-fed ecosystem in Selangor North West Project. NBWR could be disseminated by water from one rice farms to another.

High tendency of NBWR to disseminate their seeds at earlier than cultivated rice can increase the abundance of NBWR's seed bank in rice farms. Capabilities of NBWR seeds to germinate in wide range of condition making this seed bank a very serious threat to the distribution of NBWR in the future. At the same time, NBWR germinated faster than the seeded cultivated rice and thereby gain a competitive advantage over the cultivated rice. Unless, we implement good management practices in rice farms to control seed bank from germinating. Method of land preparation has an important comportment on the emergence of NBWR. Increasing soil rotovation will reduce the emergence of NBWR seedlings. Management of water in rice field influences the infestation of NBWR. Poor water management due to improper land preparation may encourage the NBWR infestation. Since the left NBWR plants are harvested with the cultivated rice due to their similarity, which the farmers again sow in the fields in the next season and retain further the problem of NBWR.