Summary

The effect of *hsp* gene transcription levels on the fitness of *Meloidogyne hapla* Chitwood, 1949, second-stage juveniles (J2) across a selection of temperature ranges

Nematodes (Nematoda) are among the most abundant metazoan taxa on Earth. They are found in almost every habitat, including soil. Of more than 284,000 named nematode species, about 1.5% occur as parasites in plants. One of the groups of parasites most harmful to crops are nematode species belonging to the genus *Meloidogyne*. As a result of feeding by these parasites the plant roots form swellings called "knots", hence they are commonly known as root-knot nematodes. In Europe, including Poland, they are represented by the northern root-knot nematode *Meloidogyne hapla*, a common pest of many dicot crops. This nematode is a dangerous parasite because it not only feeds on many species of arable crops, but it can also survive in the soil at low temperatures during winter. Two of the developmental stages: the egg state, which overwinters in soil, and the second-stage juvenile (J2) hatching from these eggs and subsequently infecting young plants at the beginning of the growing season, thus securing the survival of the nematode population, are particularly important for the survival of this species. The growth and activity of root-knot nematodes, depend, in addition to the host plant, on ambient factors, including temperature. Although many studies have been conducted on *M. hapla* and other species of the genus *Meloidogyne* into the effects of temperature on the development of the egg state as well as the J2 specimens, the body size and weight of the J2 specimens, the body lipid content in the J2 specimens, and also on the motility of this developmental stage, only a few of these studies addressed the expression of heat shock genes (*hsp* genes).

The aim of the study was to investigate the response of the *M. hapla* nematode to changes in ambient temperature. It was carried out on nematodes of this species collected from soil in Poland. The project was pursued by performing comprehensive studies at the molecular and physiological levels (whole-organism response) in the egg state and in J2 specimens, after incubation at selected temperatures and for pre-determined exposure duration times. Both stages were incubated at stress-inducing temperatures of 5°C (cold stress), 35°C, and 40°C (heat stress), and non-stress temperatures of 10°C, 20°C, and 30°C for 1, 2, 8, and 24 hours, and J2 specimens were additionally incubated for 336, 1008, and 1344 hours. For the first time in this species, the study of the effects of cold and heat stress on the levels of transcription (expression) of the *Mh-hsp*90, *Mh-hsp*1, *Mh-hsp*4, *Mh-hsp*6, *Mh-hsp*60, *Mh-*

*dnj*19, *Mh-hsp*43, and *Mh-hsp*12.2 genes was carried out in the egg state and the J2 specimens, as well as an examination of the physiological status (length, width, body weight, mass and area of stained lipids) and motility in J2 specimens. For the first time in *M. hapla*, the study covered parameters – length, width, and body weight measured in J2 specimens hatched from eggs incubated in the above-mentioned conditions of temperature and incubation duration periods.

The findings of these tests show in great detail the response to selected temperatures in both developmental stages of *M. hapla*. The stress temperature of 40°C proved to be lethal to the egg state. In contrast, the J2 specimens, depending on the exposure time, did not survive incubation at 35°C and 40°C. Studies of *hsp* genes showed their diverse responses to stress temperatures. Of the eight genes investigated, only two, i.e., *Mh-hsp*60 and *Mh-dnj*19, responded with increased expression to hot and cold stress, in both developmental stages. More *hsp* genes were found to respond to heat stress and their expression levels were higher than those triggered by cold stress. The increase in expression levels of most *hsp* genes during incubation at stress temperatures was higher in J2 specimens than in the egg state. The obtained results suggest that the *Mh-hsp*90 and *Mh-hsp*1 genes might be used as bioindicators of how nematodes of the genus *Meloidogyne* are affected by ambient conditions. Furthermore, the study indicated that J2 specimens hatched from eggs incubated at stress temperatures were characterised by lower average values of measured parameters of the physiological status than those exhibited by J2 specimens hatched at non-stress temperatures. The studies of J2 specimens found the values of measured parameters of the physiological status to be determined by the temperature and incubation time. Stress temperatures of 35°C and 40°C, along with the increasing incubation time, were found to significantly decrease the values of the tested parameters. Studies of the motility also proved stress temperatures of 5°C and 35°C to be limiting the motility of J2 specimens rendering them immotile at 40°C.

The studies of *hsp* gene expression, parameters of physiological status, and motility, have confirmed that the optimal temperatures for the growth of the *M. hapla* nematode obtained from soils of the temperate climate zone are between 20°C and 30°C.